Novel imaging aspects in the management of patients with acute coronary syndromes

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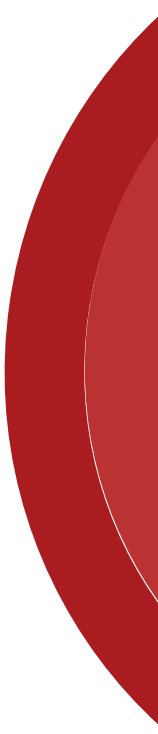

Introduction

Background

Cardiovascular diseases are responsible for 30% of all deaths worldwide and are the number one cause of deaths globally. Cardiovascular diseases caused an estimated 17.3 million deaths in 2008 [1]. Around 40% of these cardiovascular deaths are due to coronary heart disease. It is projected that coronary heart diseases will remain a leading cause of morbidity and mortality for many years to come [2].

Atherosclerosis, the main underlying pathologic process for coronary heart diseases, is a chronic disease state of the coronary arteries that slowly develops over decades before becoming clinically significant. Its pathophysiology is complex, including inflammatory and immunological events which are considered to be of central importance in the initiation and progression of atherosclerotic plaques [3,4].
Gradual chronic progression of coronary atherosclerosis may result in luminal narrowing causing symptoms of angina. However a more acute scenario exists wherein an abrupt change in plaque status causes rapid decrease in luminal patency [5].

Pivotal in these acute scenarios is the formation of thrombus, usually triggered by rupture or erosion of a vulnerable coronary plaque. Following rupture of the fibrous cap covering the atherosclerotic plaque the thrombogenic material in the core of the plaque becomes exposed to the arterial lumen. This causes platelet aggregation and the formation of thrombus. A clinically asymptomatic scenario may follow due to thrombotic sealing of the rupture. In ST-elevation myocardial infarction (STEMI) red thrombus formation often leads to acute vessel occlusion, whereas in non-ST-elevation myocardial infarction (NSTEMI) a non-occluding (mural) platelet rich thrombus is formed [6,7].

Timely diagnosis and initiation of reperfusion therapy, by means of pharmacological therapy and percutaneous reperfusion, are essential in these acute presentations of coronary artery disease in order to minimize myocardial damage [8,9]. Restoring blood flow in the infarct related artery is therefore essential, but unfortunately optimal epicardial patency does not guarantee complete and sustained reperfusion of the infarcted myocardium [10]. With an open epicardial artery, impaired flow in the microvascular compartment is predictive of myocardial infarction (MI) size, left ventricular function and mortality [11].

Monitoring simple and readily available variables such as timelines of reperfusion and epicardial and myocardial perfusion, using angiographic imaging, may aid the interventional cardiologist to identify patients who are at higher risk for future adverse events. It provides the opportunity to select the right combination of treatments for those patients in order to optimize outcomes.

An acute coronary syndrome is the most important clinical advent of atherosclerotic

disease. However, before an acute scenario occurs the slow progressing nature of atherosclerosis gives a unique opportunity in identifying the existence and the extent of atherosclerotic disease. Consequently, it allows the prospect of preventing future myocardial damage. Non-invasive imaging of the coronary arteries with computed tomography (CT) has the ability to detect coronary artery disease. Potentially this method may aid the cardiologist in search of patients with atherosclerotic disease that are most likely to develop acute coronary syndromes in the future and also identify the population in which strict primary prevention may be warranted.

Although atherosclerotic disease is likely to affect the whole coronary system, plaques prone to become unstable are not diffusely present and are only limited in number [12]. Because of its closer approach and higher resolution invasive intravascular imaging of atherosclerotic plaques may better differentiate individual plaque characteristics. Whereas CT may assist in finding a patient at risk, intravascular imaging may help in identifying the individual plaque at risk of developing future events. In this thesis we aimed to assess the value of different imaging modalities, invasive as well as non-invasive, in the evaluation and management of patients with acute coronary syndromes.

Outline of thesis

In **part 1** we focus on value of readily available monitoring variables, such as time to reperfusion, time of onset of symptoms and myocardial perfusion in patients with STEMI. With lengthening of time to reperfusion (ischemic time) the presence of microvascular perfusion decreases, resulting in increased MI size [13,14]. Timely reperfusion of the infarct-related coronary artery early after STEMI is an important factor in the improvement of outcomes. Although ischemia damages the myocardium, it is viable in part early after onset of symptoms and may be salvaged by rapid restoration of perfusion [14,15]. In previous studies, best clinical results of reperfusion by primary percutaneous intervention (PCI) were observed in patients treated within 90 to 120 minutes after symptom onset [13,14,16]. However, advances in treatment, using thrombus aspiration and pharmacological triple antiplatelet therapy during and around the PCI, have resulted in improvement of myocardial reperfusion and clinical outcomes [17-19]. These novel treatment methods may influence the time window for obtaining optimal reperfusion and clinical outcomes in STEMI patients treated by primary PCI. In **chapter 2** we studied the impact of total ischemic time on myocardial perfusion and clinical outcomes in a contemporary cohort of patients with STEMI treated with primary PCI, thrombus aspiration, and triple antiplatelet therapy

The onset of STEMI is not evenly distributed during a twenty-four hour period.

From prior research it has been known that the onset of symptoms of STEMI exhibit a circadian rhythm with peak incidence in the morning hours [20]. However, clinical outcomes seem to differ as well during the twenty-four hour period, depending on the time of onset of symptoms. Recent evidence has emerged that the time of symptom onset of STEMI is significantly associated with MI size, independent of time to reperfusion [21,22]. MI size reflects the amount of myocardial injury as a result of ischemia and is related to clinical outcomes. The time-of-day dependence of MI size also implies a circadian variation in vulnerability of the myocardium to ischemia [21,22]. This may be in turn caused by a circadian variation in myocardial perfusion. In **chapter 3** we explored if the time of symptom onset influences myocardial perfusion and its relation to MI size and subsequent outcomes in a large cohort of STEMI patients.

In **part 2** of this thesis we focus on the use of non-invasive and invasive imaging methods which may be helpful to the cardiologist in guiding cardiac interventions.

Cardiac CT has emerged as a non-invasive imaging modality allowing anatomical imaging of the heart. In particular, the potential of CT visualizing the coronary artery lumen and wall non-invasively has gained tremendous interest. Indeed, CT coronary angiography may be performed to assess coronary artery stenosis in symptomatic patients with suspected coronary artery disease [23,24]. Moreover, CT coronary angiography may potentially be useful for guiding coronary interventions and in the evaluation of the results of treatment. In addition, 3D anatomical information obtained during the examination may be clinically useful in guiding interventions of the cardiac valves or treating rhythm disorders. In **chapter 4** we provide an overview of the current applications of cardiac CT, discussing the areas in which cardiac CT may replace invasive imaging techniques and areas in which cardiac CT may be useful in guiding cardiac interventions.

About 3 to 5% [25] of patients that present as an acute MI have normal coronary arteries on invasive coronary angiography (ICA). This group of patients with acute myocardial infarction and angiographically normal coronary arteries is broadly recognized and described in several series. The pathogenetic mechanisms that cause AMI in these patients are unknown. Outward remodeling of rupture prone atherosclerotic plaques without visible luminal narrowing on ICA have been suggested as underlying cause[26]. Other proposed mechanisms are coronary dissection, endothelial dysfunction, embolism and vasospasm. In **chapter 5** we aimed to assess the presence and characteristics of atherosclerotic plaques on CT coronary angiography in patients with acute MI who have a completely normal coronary angiogram on ICA.

CT coronary angiography enables visualization of the vessel wall offering valuable information about the burden of coronary artery disease. This may provide prognostic

information as well as ability to assess the disease process. Identification of circulating immune-inflammatory markers that are associated with the atherosclerotic disease process in coronary arteries may provide additive information. The relation of neutrophil counts, neutrophil/lymphocyte ratio and other immune-inflammatory markers, with plaque burden assessed by CT coronary angiography was investigated in **chapter 6**.

Rupture or erosion of a coronary plaque containing a large necrotic/lipid core and/or a thin fibrous cap resulting in luminal thrombosis have been linked to the development of coronary events [7]. In preliminary studies with CT coronary angiography it has been observed that the presence of non-calcified plaque component is associated with the development of coronary events [27]. Accordingly, it has been speculated that non-calcified tissue containing plaque on CT coronary angiography might represent rupture prone plaques [28]. However, the ability of CT coronary angiography to discriminate between components of non-calcified plaque is limited and remains challenging [29]. Invasive coronary plaque characterization may be performed by optical coherence tomography (OCT) and intravascular ultrasound (IVUS) with high spatial resolution. Coronary artery lumen and the presence of thrombus can be accurately observed on OCT as compared to histology [30]. Moreover, a good correlation between geometrical and compositional coronary plaque characteristics has been observed between CT coronary angiography and IVUS in patients presenting with stable angina pectoris [31,32]. Nevertheless, to date no data are available on to which extent coronary artery thrombus contributes to the amount of non-calcified tissue of the plaques on CT coronary angiography in patients presenting with NSTEMI. In **chapter 7** we investigated the ability of CT coronary angiography and IVUS to differentiate between characteristics of the culprit lesion in vivo with OCT serving as the standard of reference.

During PCI microembolization of fragmented atherothrombotic material from the culprit lesion may cause additional myocardial damage [33]. Manual thrombus aspiration (TA) is effective in retrieving atherothrombotic debris [19,34]. The systematic use of TA in STEMI patients is associated with less distal embolization, an improved post-procedural myocardial perfusion and improved prognosis at 1-year follow-up [19,35]. Thus far, TA studies have been focusing mainly on patients with STEMI. One small study performed in patients presenting with NSTEMI who underwent PCI within twenty-four hours after clinical presentation demonstrated that TA is associated with high rates of retrieval of thrombotic material [34]. Whereas TA is frequently performed in patients with visible thrombus on ICA, thrombus is often not detectable on ICA [19,34]. We investigated the efficacy of TA, evaluated with OCT in patients presenting with NSTEMI after initial conservative therapy, which is described in **chapter 8**.

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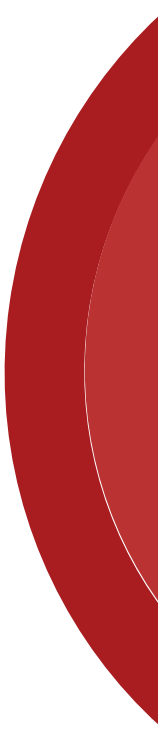

Quantitative analysis of the impact of total ischemic time on myocardial perfusion and clinical outcome in patients with ST-elevation myocardial infarction

2

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Abstract

Early reperfusion of the infarct-related coronary artery is an important issue in improvement of outcome after ST-segment elevation myocardial infarction (STEMI). In this study, the clinical significance of total ischemic time on myocardial reperfusion and clinical outcomes was evaluated in patients with STEMI treated with primary percutaneous coronary intervention and thrombus aspiration and additional triple anti-platelet therapy. Total ischemic time was defined as time from symptom onset to first intracoronary therapy (first balloon inflation or thrombus aspiration). All patients with STEMI treated with primary percutaneous coronary intervention with total ischemic times ≥30 minutes and <24 hours from 2005 to 2008 were selected. Ischemic times were available in 1,383 patients, of whom 18.4% presented with total ischemic times ≤2h, 31.2% >2 to 3 hours, 26.8% >3 to 5 hours and 23.5% >5 hours. Increased ischemic time was associated with age, female gender, hypertension, and diabetes. Patients with total ischemic time <5 hours more often had myocardial blush grade 3 (40% to 45% vs 22%, p<0.001) and complete ST-segment resolution (55% to 60% vs 42%; p = 0.002) than their counterparts with total ischemic times >5 hours. In addition, patients with total ischemic times ≤ 5 hours had a lower 30-day mortality (1.5% vs 4.0%; p = 0.032) than patients with total ischemic times >5 hours. In conclusion, in this contemporary cohort of patients with STEMI treated with primary percutaneous coronary intervention, triple anti-platelet therapy, and thrombus aspiration, short ischemic time is associated with better myocardial reperfusion and decreased mortality. After a 5-hour period in which outcomes remain relatively stable, myocardial reperfusion becomes suboptimal and mortality increases.

Introduction

Early reperfusion of the infarct-related coronary artery is an important issue in the improvement of outcomes after ST-elevation myocardial infarction (STEMI). Although the myocardium is damaged during ischemia, it is viable in part early after symptom onset and may be salvaged by rapid reperfusion.[1,2] The presence of microvascular obstruction increases with longer ischemic times, resulting in an increased infarct size.[2,3] In previous studies the best clinical results of reperfusion by primary percutaneous coronary intervention (PCI) have been observed in patients treated within 90 to 120 minutes after symptom onset.[2-5] Pretreatment with aspirin, heparin and clopidogrel before hospital admission and the administration of a glycoprotein IIb/IIIa inhibitor during primary PCI is associated with improvements in myocardial reperfusion and clinical outcomes.[6-8] Furthermore, it has been demonstrated that thrombus aspiration results in an additional improvement of myocardial reperfusion.[9,10] The application of these innovative pharmacologic and intracoronary treatment strategies could influence the time window to obtain optimal reperfusion and clinical outcomes by primary PCI in patients with STEMI. The aim of this study was to evaluate the impact of total ischemic time on myocardial reperfusion and clinical outcomes in a large contemporary cohort of patients with STEMI treated with primary PCI, with thrombus aspiration, and triple anti-platelet therapy.

Methods

We performed an analysis of ischemic time data from consecutive patients with STEMI presenting to the University Medical Center of Groningen from January 2005 to July 2008. Inclusion criteria were symptoms of chest pain suggestive for acute myocardial infarction lasting ≥30 minutes and <24 hours before hospital admission, electrocardiographic findings of ST-segment elevation >0.1 mV in ≥2 leads, and the performance of a primary PCI procedure. Exclusion criteria were the presence of cardiogenic shock and the existence of a life-threatening disease with a life expectancy of <6 months. Patients treated with acute coronary artery bypass grafting after primary PCI were not enrolled. The University Medical Center of Groningen provides 24-hours emergency cardiac care 7 days a week. It is situated in a region with 750,000 inhabitants and has 7 referral hospitals. When acute coronary syndromes are suspected, 12-lead electrocardiography is performed and the results interpreted by the ambulance physician, aided by a computer algorithm and feedback after fax transmission from our coronary care unit. After confirmation of STEMI, the STEMI treatment protocol is initiated. This includes that the coronary care unit of our center is contacted and informed about the arriving patient, and direct activation

of the cardiac catheterization team. The patient is directly transported to the catheterization laboratory, thereby bypassing other regional hospitals. In our region, ambulance transfer times vary, to a maximum of 30 minutes. The STEMI protocol has been initiated in January 2004 and has remained unchanged during the period under study.

All patients were treated with aspirin (500 mg), heparin (5,000 IU) and clopidogrel (600 mg) after confirmation of ST-segment elevation on the first electrocardiogram, usually obtained in the ambulance before hospital admission. During primary PCI, patients received the glycoprotein IIb/IIIa inhibitor abciximab (0.25 mg/kg intravenously) if not contraindicated. Additional heparin was administered during procedure, guided by the activated clotting time. As the initial step during primary PCI, manual thrombus aspiration was performed in about half of the patients until 2006. After 2006, thrombus aspiration was performed in all patients whenever possible. After restoration of flow through the infarct-related lesion, a stent was implanted. Balloon pre- and postdilatation were used when necessary to achieve visualization of the infarct-related lesion before stent placement or optimal stent deployment. After primary PCI, patients received aspirin, clopidogrel (>1 month), β blockers, lipid-lowering agents, and angiotensin-converting enzyme inhibitors or angiotensin II receptor blockers.[11]

Total ischemic time was defined as the time from symptom onset to the first intracoronary therapy (first balloon inflation or thrombus aspiration). Information on the time of symptom onset was systematically collected by asking the patient or his relatives about initiation of continuous chest pain before hospital admission.

Angiographic records before and after primary PCI were evaluated by 2 experienced observers blinded for clinical data. On the initial angiogram and on the final angiogram, Thrombolysis In Myocardial Infarction (TIMI) flow grade, angiographic evidence of thrombus in the infarct-related lesion, and distal embolization were assessed.[12-14] In addition, myocardial blush grade (MBG) was assessed on the angiogram after stenting.[15] The 12-lead electrocardiograms obtained at presentation and 30 to 60 minutes after primary PCI were evaluated by 2 experienced observers blinded to angiographic and clinical data. ST-segment elevation resolution and the presence of Q waves were assessed.[16,17] Aspirated material was collected and analyzed for patients from 2005 to 2006. Thrombus aspiration was defined as effective when atherothrombotic material was present in the aspirated samples.

Follow-up data at 30 days after primary PCI were collected from hospital records, written questionnaires, and telephone interviews. We report all-cause mortality. Reinfarction was defined as the onset of recurrent symptoms of ischemia combined

with new ST-segment elevations and/or a second increase of serum creatine kinase or creatine kinase-MB to ≥2 times the upper limit of the normal range. Target vessel revascularization was defined as PCI or bypass grafting of the infarct-related coronary artery.

The primary end point of our study was optimal myocardial reperfusion, defined as an MBG of 3 and/or ST-segment resolution >70%. Secondary end points were the presence of new Q waves on electrocardiography after primary PCI, enzymatic infarct size as assessed by the maximum creatine kinase-MB level, and mortality, reinfarction and target vessel revascularization at 30 days after primary PCI.

Patients were classified in 4 time categories of whole hours according to total ischemic time, approaching a distribution in quartiles. Categorical variables are presented as frequency values and proportions, and differences between ischemic time categories were evaluated using chi-square or Fisher's exact tests. Continuous variables with normal distributions are presented as mean ± SD, whereas variables with non-normal distribution are presented as medians with interquartile ranges. Differences in continuous variables between ischemic time categories were evaluated using 1-way analysis of variance or the Kruskal-Wallis nonparametric test as appropriate. The cumulative incidence of clinical endpoints was evaluated by the method of Kaplan and Meier, and differences in cumulative event rates according to ischemic time were evaluated using log-rank tests. Univariate and multivariate logistic regression analyses were applied to study the relation between ischemic time and the primary end point, myocardial reperfusion, assessed as MBG of 3 and ST-segment resolution >70%. In multivariate analysis, we adjusted for potential confounders associated with the end points in univariate analysis. We report crude and adjusted odds ratios together with corresponding 95% confidence intervals. For all analyses, 2-sided p values < 0.05 were defined as significant. Statistical analysis was performed using the SPSS version 16.0 (SPSS, Inc., Chicago, Illinois).

Results

From January 2005 to July 2008 1,731 consecutive patients with STEMI were treated with primary PCI at our hospital (Figure 1). Ischemic time was available in 1,383 patients, (79.9%) of all 1,731 STEMI patients. Of these, 255 patients (18.4%) had ischemic times ≤2hours, 432 patients (31.2%) had times >2 to 3 hours, 371 patients (26.8%) had times >3 to 5 hours and 325 patients (23.5%) had times >5 hours. The median ischemic time was 3.1 hours (interquartile range 2.3 to 4.8). As listed in Table 1, prolonged ischemic time was associated with age, female gender, hypertension, diabetes, and smoking status. Angiographic and procedural characteristics are listed

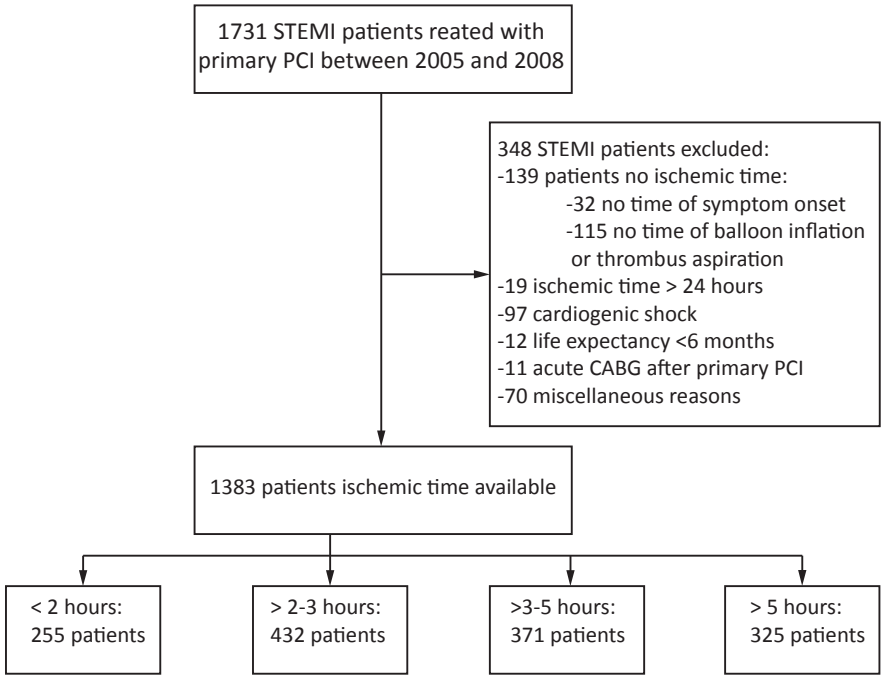


Figure 1. Flow diagram. Flow diagram of the inclusion of STEMI patients with primary PCI

in Table 2. Ischemic time was associated with multivessel disease, the presence of collateral arteries, the use of glycoprotein IIb/IIIa inhibitors, balloon dilatation, and stent implantation. The incidence of TIMI grade 3 flow decreased from 93.3% to 79.9% after 5 hours ($p<0.001$).

MBG was analyzed in 1,358 patients (98.2%) after primary PCI. In the first 5 hours of ischemic time, MBG of 3 varied from 44.6% and 40.1%, but decreased to 22.3% after 5 hours ($p<0.001$; Table 3, Figure 2). ST-segment resolution could be analyzed in 1,243 patients (89.9%). Complete ST-segment resolution varied from 54.5% and 59.8% in the first 5 hours but decreased to 42.4% after 5 hours ($p<0.001$; Table 3, Figure 2). Multivariate analysis showed that after correction for predictive baseline and procedural variables in the univariate analysis, ischemic time was a significant predictor of MBG of 3 and ST-segment resolution >70% (Table 4). In addition, the presence of Q waves and the maximum value of creatine kinase-MB were associated with prolonged total ischemic time (Table 3, Figure 2). A total of 29 patients (2.1%) had died at 30 days after primary PCI. Mortality was about 1.5% up to 5 hours of ischemic time and increased to 4.0% after 5 hours ($p = 0.032$; Table 3). Figure 3 shows differences in cumulative rates of mortality at 30 days ($p = 0.05$).

Table 1. Baseline characteristics

Variable	Myocardial Ischemic Time (hours)				p Value
	≤2 h (n = 255)	>2-3 h (n = 432)	>3-5 h (n = 371)	> 5 h (n = 325)	
Ischemic time (hours)	1.83 (1.50-1.92)	2.50 (2.25-2.75)	3.75 (3.33-4.25)	7.25 (5.87-10.58)	<0.001
Age (years)	61.6 ± 12.0	61.9 ± 12.2	62.5 ± 13.0	65.2 ± 12.6	0.001
Men	193/255 (75.7%)	321/432 (74.3%)	266/371 (71.7%)	215/325 (66.2)	0.006
Hypertension	84/249 (33.7%)	143/411 (34.8%)	118/364 (32.4%)	139/316 (44.0)	0.022
Hypercholesterolemia	66/217 (30.4%)	98/357 (27.5%)	88/315 (27.9%)	80/280 (28.6)	0.763
Diabetes mellitus	18/254 (7.1%)	44/426 (10.3%)	34/368 (9.2%)	50/323 (15.5)	0.003
Myocardial infarction	18/254 (7.1%)	42/426 (9.9%)	36/368 (9.8%)	31/323 (9.6)	0.392
Previous PCI	18/254 (7.1%)	28/427 (6.6%)	25/366 (6.8%)	20/322 (6.2)	0.735
Previous CABG	4/255 (1.6%)	9/427 (2.1%)	15/368 (4.1%)	10/323 (3.1)	0.115
Current smoker	135/231 (58.4%)	199/397 (50.1%)	175/337 (51.9%)	133/293 (45.4)	0.011

Data are expressed as median (interquartile range), as mean ± SD, or as number (percentages)
CABG = coronary artery bypass grafting

Discussion

In this contemporary cohort of patients with STEMI treated with primary PCI, thrombus aspiration, and triple anti-platelet therapy, myocardial reperfusion, as assessed by angiography (MBG of 3) and electrocardiography (ST-segment resolution >70%), was better in patients with total ischemic times ≤5 hours than in those with longer ischemic times. Interestingly, if ischemic times can be limited to ≤5 hours, the duration of the ischemia seems to only modestly influence myocardial reperfusion. Most patients with STEMI could be treated with primary PCI in the first 5 hours after symptom onset. Treatment within these golden hours of primary PCI results in better myocardial reperfusion and clinical outcomes.

Our findings confirm the observation in various previous studies that prolonged total ischemic time is associated with impaired myocardial reperfusion.[18,19] Recent studies have also confirmed that time to reperfusion is associated with infarct size as assessed using technetium sestamibi imaging and magnetic resonance imaging,[2,3,5] with treatment benefit especially in patients who were treated within <90 to 120 minutes after symptom onset.[2,5] In addition, microvascular obstruction increased over time.[2,3]

A relation between treatment delay and mortality was clearly described by Boersma et al,[20] showing that in patients with STEMI treated with fibrinolytic therapy, relative as well as absolute mortality reduction was significantly higher when fibrinolysis was performed in the first 2 hours after symptom onset than after that time. In patients treated with primary PCI, this observation was confirmed, often showing that patients treated within the first 2 hours had a better prognosis than patients treated after 2 hours.[4,18,19,21] However, these studies did not systematically use triple anti-platelet therapy and did not perform thrombus aspiration in addition to primary

Table 2. Angiographic and procedural characteristics

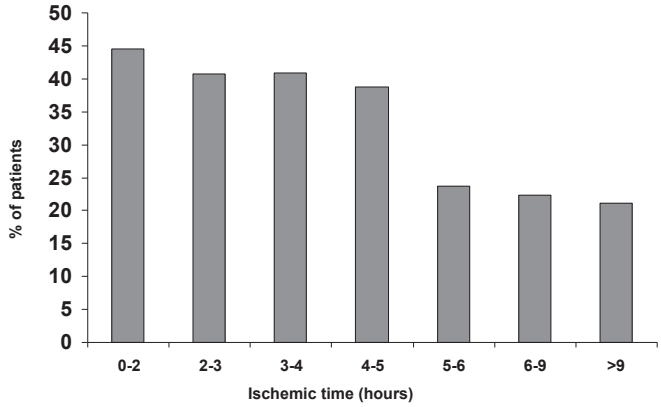
Variable	Myocardial Ischemic Time (hours)				p Value
	≤2 h (n = 255)	>2-3 h (n = 432)	>3-5 h (n = 371)	> 5 h (n = 325)	
Pre-PCI angiography					
Anterior infarction	113/255 (44.3%)	184/432 (42.6%)	152/371 (41.0%)	141/325 (43.4%)	0.763
Multivessel disease	156/254 (61.4%)	275/431 (63.8%)	240/371 (64.7%)	226/324 (69.8%)	0.036
Collateral arteries	65/252 (25.8%)	95/420 (22.6%)	91/362 (25.1%)	108/320 (33.8%)	0.011
Thrombus before PCI	141/252 (56.0%)	236/425 (55.5%)	211/364 (58.0%)	181/321 (56.4%)	0.787
TIMI grade 0 or 1 flow before PCI	152/253 (60.1%)	244/428 (57.0%)	221/369 (59.9%)	211/323 (65.3%)	0.096
Procedural					
Thrombus aspiration	149/252 (59.1%)	265/431 (61.5%)	218/366 (59.6%)	189/324 (58.3%)	0.648
Effective Glycoprotein IIb/IIIa inhibitor	60/80 (75.0%)	116/158 (73.4%)	108/153 (70.6%)	86/122 (70.5%)	0.401
Balloon dilatation	239/253 (94.5%)	402/430 (93.5%)	343/369 (93.0%)	291/325 (89.5%)	0.022
Stent implantation	123/243 (50.65%)	216/415 (52.0%)	203/352 (57.7%)	211/312 (67.6%)	<0.001
Intra-aortic balloon pump	231/243 (95.1%)	397/419 (94.7%)	330/352 (93.8%)	284/311 (91.3%)	0.047
Post-PCI angiography	6/150 (4.0%)	14/288 (4.9%)	14/261 (5.4%)	15/216 (6.9%)	0.199
TIMI grade 3flow after PCI	236/253 (93.3%)	385/430 (89.5%)	321/370 (86.8%)	259/324 (79.9%)	<0.001
Distal embolization after PCI	15/233 (6.4%)	33/389 (8.5%)	21/348 (6.0%)	23/300 (7.7%)	0.925
Thrombus post PCI	3/253 (1.2%)	6/431 (1.4%)	10/370 (2.7%)	9/323 (2.8%)	0.082

Data are expressed as number (percentages).

PCI. In addition, patients with prolonged ischemic time (12 to 24 hours) were often not included, but may still benefit from primary PCI.[22]

In the present study, myocardial reperfusion decreased after 5 hours of total ischemic time, which was a considerably longer ischemic time than reported in previous studies. First, it could be suggested that the administration of aspirin, heparin and clopidogrel before hospital admission has a favourable effect on reperfusion. Because they are already administered during the ischemic time period, an open infarct-related artery may be present in a considerable portion of patients before primary PCI.[23] Second, the administration of glycoptroteïn IIb/IIIa inhibitors during primary PCI may result in reduced platelet aggregation and microthrombi, which otherwise would increase as total ischemic time prolongs. Third, the performance of thrombus aspiration may have improved myocardial reperfusion, because it is still feasible and effective when total ischemic time increases. In the first 5 hours, the duration of ischemia seems to only modestly influence myocardial reperfusion. However, we suggest that all patients should be treated with primary PCI as soon as possible, independent of time of symptom onset. First, this will lower the median total ischemic time and decreases the number of patients treated after 5 hours. Second, it shortens the time interval during which life-threatening arrhythmias and cardiogenic shock can develop.

A



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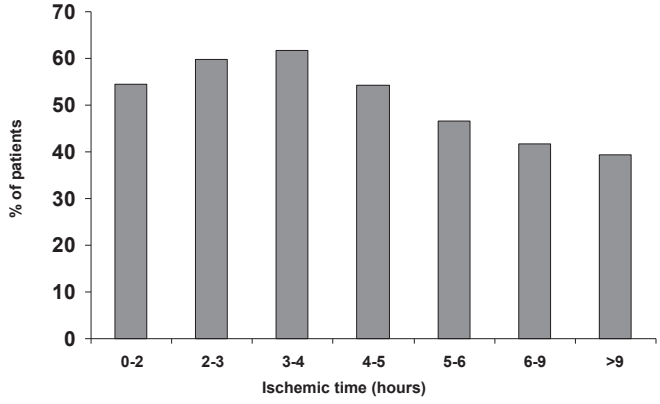

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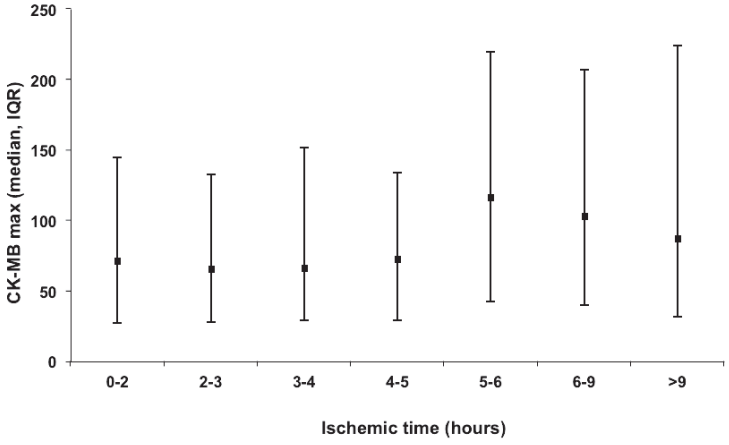

Figure 2A. **Myocardial blush grade 3 after primary PCI** Myocardial reperfusion as assessed by myocardial blush grade 3. B. **ST-segment resolution >70% after primary PCI.** Myocardial reperfusion as assessed by complete ST-segment resolution. C. **CK-MB maximum after primary PCI** Infarct size as assessed by CK-MB maximum.

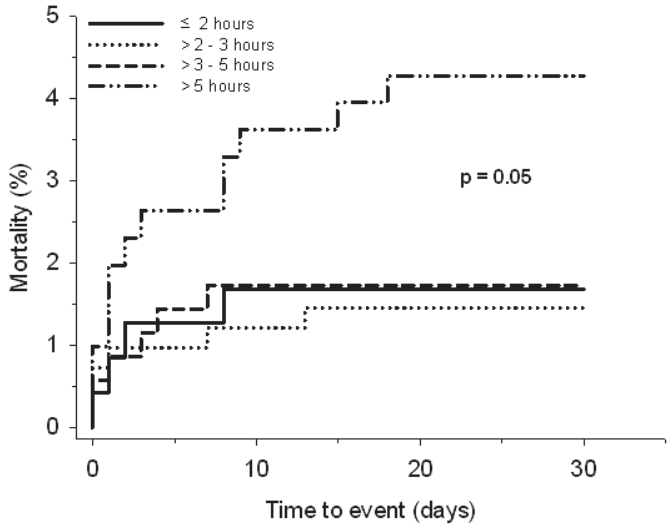

Figure 3. **Kaplan Meier curve 30 day mortality** Kaplan Meier curve for 30 day mortality for the 4 intervals of ischemic time.

Total ischemic time was associated with mortality at 30 days after primary PCI, and mortality rates were nearly 3 times higher in patients with an ischemic time >5 hours than in patients presenting in the first 5 hours. However, it should be considered that patients presenting late were older, more often had cardiovascular risk factors, more often had multivessel disease, less often were administered glycoprotein IIb/IIIa inhibitors, more often underwent balloon dilatation, and less often underwent stent implantation. These differences in patient baseline characteristics may in part explain the increased mortality after 30 days.

As total ischemic time increases, platelet aggregation continues, and blood stagnation causes occlusion of the coronary artery.[24] Although the prevalence of total coronary occlusion did not significantly increase with prolonged ischemic time, balloon dilatation was more often performed in patients with increased ischemic time. The additional use of balloon dilatation may have contributed to fragmentation of thrombus material and a higher incidence of embolization into the microvasculature.

In this contemporary cohort patients with STEMI were treated with aspirin, heparin and clopidogrel, the glycoprotein IIb/IIIa inhibitor abciximab, and thrombus aspiration. In recent years, new antithrombin treatments and new glycoprotein IIb/IIIa inhibitors have been developed. The use of bivalirudin has been investigated, with promising results, especially on bleeding complications.[25,26] The glycoprotein IIb/IIIa inhibitor eptifibatide was suggested to be as effective as abciximab in restoring myocardial perfusion.[27] In addition, 2 new thienopyridines, prasugrel and ticagrelor, have become available, causing a more rapid and higher level of platelet inhibition and

Table 3. Outcome characteristics

Variable	Myocardial Ischemic Time (hours)				p Value
	≤2 h (n = 255)	>2-3 h (n = 432)	>3-5 h (n = 371)	> 5 h (n = 325)	
Myocardial reperfusion					
MBG 3	112/251 (44.6%)	173/425 (40.7%)	146/364 (40.1%)	71/318 (22.3%)	<0.001
ST-segment resolution > 70%	126/231 (54.5%)	234/391 (59.8%)	197/333 (59.2%)	122/288 (42.4%)	0.002
Measures of infarct size					
Q waves	186/238 (78.2%)	315/403 (78.2%)	268/343 (78.1%)	251/295 (85.1%)	0.047
Creatine kinase -MB maximum (U/L)	71.0 (26.9-145.0)	65.5 (28.0-132.8)	68.9 (29.1-147.7)	98.2 (36.0-222.3)	<0.001
Clinical outcome at 30 days					
Mortality	4/255 (1.6%)	6/430 (1.4%)	6/371 (1.6%)	13/325 (4.0%)	0.032
Reinfarction	4/255 (1.6%)	3/430 (0.7%)	6/371 (1.6%)	5/325 (1.5%)	0.647
Target vessel revascularization	11/255 (4.3%)	14/430 (3.3%)	22/371 (5.9%)	19/325 (5.8%)	0.134

Data are expressed as number (percentages) or as median (interquartile range).

improving clinical outcome.[28,29] When they are added to or replaced by clopidogrel in the prehospital treatment, it could be suspected that these thienopyridines lead to more intitial patency of the infarct-related vessel.

Several limitations should be considered. This was a single-center study, and therefore data can not automatically be extrapolated to other PCI centers, although with the inclusion of 80% of all patients with STEMI, this patient cohort does reflect a real-world clinical practice. However, it should be mentioned that patients presenting with cardiogenic shock were excluded in our analysis, which may have influenced our results. In patients with cardiogenic shock it was often not possible to assess the onset of ischemic time. Ischemic time data were not available for all patients because of missing times of symptom onset, thrombus aspiration or balloon inflation. The exclusion of these patients may have influenced our results, although the baseline characteristics of the included and excluded patients were similar. Furthermore, thrombus aspiration was performed in only 60% of patients. However, the incidence of thrombus aspiration did not differ between the ischemic time groups. In addition, ST- segment resolution could only be analyzed in 90% of patients, because the electrocardiograms at presentation or after primary PCI were not available, or because of the occurrence of an intraventricular conduction delay. Furthermore, maximum creatine kinase-MB levels were measured only during the stay in our hospital. Because some patients were transferred to a regional hospital 1 day after primary PCI, maximum measured creatine kinase-MB may have been too low.

Table 4. Univariable and multivariable analysis between total ischemic time and myocardial reperfusion, assessed by myocardial blush grade 3 and ST-segment resolution >70%.

Variable	Univariate analysis		Multivariate analysis	
	OR*	95% CI	OR*	95% CI
MBG 3				
Ischemic time ≤ 2 hours	2.80	1.95-4.03	2.66	1.76-4.03
Ischemic time 2-3 hours	2.39	1.72-3.31	2.33	1.60-3.38
Ischemic time 3-5 hours	2.33	1.66-3.26	2.31	1.57-3.39
Age	0.97	9.96-0.98	0.98	0.97-0.99
Current smoker	1.35	1.07-1.70	1.10	0.85-1.43
Multivessel disease	0.79	0.63-0.99	1.10	0.85-1.44
Collateral ateries	0.66	0.49-0.89	0.80	0.60-1.08
Glycoprotein IIb/IIIa inhibitor	1.65	1.04-2.60	1.08	0.62-1.87
Balloon dilatation	0.41	0.33-0.52	0.48	0.37-0.61
ST-segment resolution > 70%				
Ischemic time ≤ 2 hours	1.63	1.15-2.31	1.14	0.77-1.68
Ischemic time 2-3 hours	2.03	1.49-2.76	1.68	1.19-2.38
Ischemic time 3-5 hours	1.97	1.43-2.71	1.71	1.20-2.44
Age	0.98	0.97-0.99	0.99	0.98-1.00
Diabetes	0.56	0.39-0.81	0.64	0.42-0.98
Current smoker	1.30	1.03-1.65	1.19	0.92-1.54
Multivessel disease	0.71	0.56-0.90	0.83	0.63-1.08
Collateral arteries	0.69	0.54-0.89	0.80	0.60-1.06
Balloon dilatation	0.58	0.46-0.74	0.64	0.50-0.83
Stent implantation	1.62	1.01-2.59	1.39	0.82-2.36

CI = confidence interval; OR = odds ratio.
* For total ischemic time, the reference categoriy was ischemic time group >5 hours.

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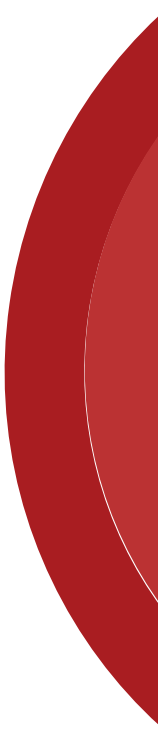

Time of symptom onset and value of myocardial blush and infarct size on prognosis in patients with ST-Elevation Myocardial Infarction

3

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Abstract

In patients with ST-segment elevation myocardial infarction (STEMI) the time of onset of ischemia has been associated with myocardial infarction (MI) size. Myocardial blush grade (MBG) reflects myocardial response to ischemia/reperfusion injury, which may differ according to time of day. The aim of our study was to explore the 24-hour variation in MBG and MI size in relation to outcomes in STEMI patients. A retrospective multicenter analysis of 6970 STEMI patients was performed. Time of onset of STEMI was divided into four 6-hour periods. STEMI patients have a significant 24-hour pattern in onset of symptoms, with peak onset around 09:00 hour. Ischemic time was longest and MI size, estimated by peak creatine kinase concentration, was largest in patients with STEMI onset between 00:00 and 06:00 hour. Both MBG and MI size were independently associated with mortality. Time of onset of STEMI was not independently associated with mortality when corrected for baseline and procedural factors. Interestingly, patients presenting with low MBG between 00:00 and 06:00 had a better prognosis compared to other groups. In conclusion, patients with symptom onset between 00:00 and 06:00 hour have longer ischemic time and consequently larger MI size. However, this does not translate into a higher mortality in this group. In addition, patients with failed reperfusion presenting in the early morning hours have better prognosis, suggesting a 24-hour pattern in myocardial protection.

Introduction

Cardiovascular physiological and biological factors exhibit circadian rhythms, which influence cardiovascular diseases [1,2,3]. The onset of acute coronary events, such as acute myocardial infarction (MI), sudden cardiac death and stent thrombosis, has a profound peak incidence in the morning hours [4,5,6,7]. Circadian variations in intrinsic factors such as adrenergic activity and thrombogenicity, have been proposed as important contributing factors of this time-of-day dependence [8,9].

In ST-elevation myocardial infarction (STEMI) patients, MI size reflects the amount of myocardial injury. Restoring myocardial blood flow, by means of primary percutaneous coronary intervention (PCI), positively influences the myocardial response to injury. Recent studies show a significant association of time of symptom onset with MI size, demonstrating larger MI size in the early morning hours, consequently resulting in a reduction of left ventricular function and increased incidence of heart failure [10,11,12]. The time-of-day dependent variation of MI size implies also a circadian variation in (intrinsic) vulnerability of the myocardium to ischemia [10,12].

Myocardial blush grade (MBG) represents an angiographic measurement of microvascular (capillary) perfusion. It reflects myocardial response to ischemic injury and reperfusion and is associated with both short and long term outcomes after acute MI [13,14]. A time-of-day variation to ischemic injury may therefore be reflected in a circadian variation of MBG.

The aim of our study was to evaluate if the time of symptom onset influences the MBG and MI size in relation to outcomes in a cohort of STEMI patients. We hypothesize that there is a time of symptom onset dependent relation of MBG to MI size resulting in differences in outcomes.

Materials and methods

Study population

We performed retrospective analysis of consecutive patients admitted with STEMI to two large PCI centers (University Medical Center Groningen, Groningen and Isala Clinics, Zwolle) in the Netherlands. Patients who presented in the period from January 2004 to December 2010 were included. The study was approved by the local ethics committee and conformed to the ethical standards outlined by Portaluppi et al. [15].

Uniform ambulance protocols involving all patients with symptoms suspect for STEMI are used in both hospitals. All patients were transported to the catheterization laboratory, and acute coronary angiography and subsequent primary PCI were performed as part of the routine treatment for all STEMI patients. The strategy of intervention (e.g. balloon dilatation, thrombus aspiration, stent placement) was left

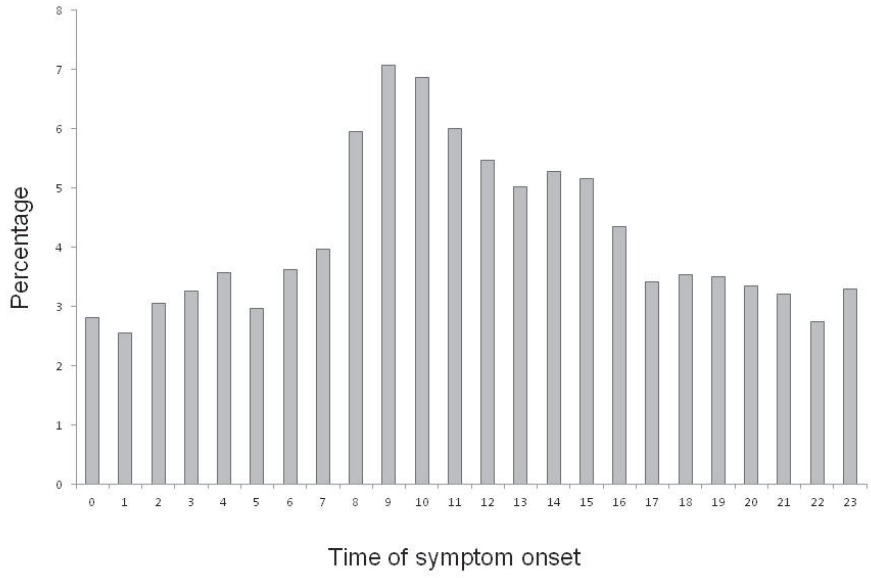


Figure 1. Percentages of patients with time of symptom onset per hour group - Percentage of patients per hour group. 0 = 00:00 – 01:00 hour; 1= 01:00 – 02:00 hour; 2 = 02:00 – 03:00; etc.

to the operator's discretion. This protocol is in accordance with the guidelines of the European Society of Cardiology, American College of Cardiology and the American Heart Association [16,17].

STEMI was defined as the presence of chest pain suggestive of myocardial ischemia with ECG signs compatible with acute MI (ST-segment elevation ≥2mm in V_2-V_3 and ≥1mm in all other leads) [18]. All patients received pretreatment with acetylsalicylic acid (500mg intravenously), heparin (5000IU), and clopidogrel (300 or 600mg orally, according to at that time valid guidelines) during transportation to the hospital, or these drugs were administered at the emergency ward in case of patient's self-referral.

All patients of whom time of symptom onset was known and who were treated by PCI within 12 hours after symptom onset, as stated in international guidelines [16,17], were included in the analyses.

Data collection

Patient characteristics were recorded on admission in either case record forms or a computer-based database. The primary independent variable was time of symptom onset, which is defined as the self reported symptom onset time. To gain insight in the 24-hour pattern of complaints of the patients, 1-hour intervals were selected to perform figurative analysis (Figure 1). Subsequently, patients were divided into four

groups according to the time of symptom onset: Group 1: 00:00–06:00 hour; group 2: 06:00–12:00 hour; group 3: 12:00–18:00 hour; and group 4: 18:00–00:00 hour. This is in accordance with recent literature [10,12,19].

Pre-hospital delay was defined as the time form onset of chest pain to the time of arrival at the hospital (door time point). Door to balloon time was defined as the time from arrival at the hospital to the restoration of epicedial blood flow by first intervention (stenting, balloon dilatation of thrombus aspiration) during primary PCI. Ischemic time was defined as the time from onset of chest pain to the restoration of epicardial blood flow by first intervention during primary PCI. Vital signs (systolic and diastolic blood pressure and heart rate) were collected at arrival in the catheterization laboratory. Epicardial reperfusion was assessed according to the Thrombolysis In Myocardial Infarction (TIMI) flow grading system before and after PCI [20]. Microvascular reperfusion was assessed by MBG as follows (14): 0=no myocardial blush, or contrast density; 1=minimal myocardial blush; 2=moderate myocardial blush but less than that obtained during angiography of a contralateral or ipsilateral non-infarct-related coronary artery; and 3=normal myocardial blush comparable to that obtained during angiography of a contralateral or ipsilateral non-infarct-related coronary artery. Procedural success was defined as TIMI flow 3 with MBG 2 to 3 after PCI [21]. MI size was estimated by peak levels of serum creatine kinase (CK) and myocardial-band of CK (CKMB). CK and CKMB measurements were collected in all patients following a standardized protocol at admission and 3, 6, 12 and 24 hours after primary PCI. Marker levels of CK and CKMB were determined as a UV assay and immunologic UV assay (Mega, Merck, Darmstadt, Germany) from January 2004 till March 2006 and thereafter as a UV assay and an immunologic UV assay (Modular P, Roche, Mannheim, Germany) in Groningen and as a UV assay and an immunologic UV assay (Modular P, Roche, Mannheim, Germany) in Zwolle. Clinical follow-up data were collected from hospital records, telephone contact (with either the general practitioner or the patient) and through coupling of municipal mortality records. Reinfarction was defined as the onset of symptoms of ischemia combined with new ST-segment elevations or an increase of serum CK of CKMB to at least twice the upper limit of the normal range. Major adverse cardiac event was defined as death, reinfarction or target vessel revascularization (either by PCI or coronary artery bypass grafting).

Statistical analysis

Normally distributed continuous variables are presented as mean±SD, unless otherwise specified. Continuous variables with skewed distribution are presented as

medians with interquartile range (Q1 and Q3). Categorical variables are presented as numbers and percentages. Group differences were tested using Kruskal-Wallis, ANOVA, log- rank and Pearson χ^2 tests where appropriate.

The distribution of time of symptom onset over 24 hours was tested against the null hypothesis of a uniform distribution with the Rayleigh test [22]. Multivariable Cox regression analysis was fitted to analyze the predictive value of MI size and MBG on all-cause mortality. Baseline and procedural variables: time of symptom onset, age, gender, history of diabetes, systolic blood pressure, heart rate and ischemic time were selected for both models beforehand. Time of symptom onset was modeled using sinusoidal functions. The four-degree of freedom sinusoidal function consisted of 1-period sine, 1-period cosine, 2-period sine and 2-period cosine variables [19]. The likelihood ratio test was used to evaluate the statistical significance of time of symptom onset. Ischemic time and CK were first transformed logarithmically, to account for its skewed distribution. Retransformation of parameter and 95% confidence intervals into meaningful units was performed in the final model. The Kaplan-Meier method was used for survival analyses for groups categorized in low/high MBG (MBG 0/1 vs. MBG 2/3) and above/below median peak CK levels. Group differences were tested using the logrank test. Logistic regression analysis was fitted to calculate odds ratios for each group stratified by high/low MBG and above/below median peak CK levels. The group with lowest mortality was used as a reference category. The bonferroni method was used to adjust *p* values for multiple testing. Statistical significance was defined as *p* value of <0.05. Statistical analyses were performed using STATA version 11 (College Station, TX).

Results

From January 2004 until December 2010, a total of 7850 patients with STEMI treated with primary PCI were included: 3360 in Groningen and 4490 in Zwolle. Time of onset of complaints was unknown in 282 patients, and 598 of the remaining patients had time from symptom onset to first intervention of >12 hours. Therefore, 6970 patients were included in the final analyses. MBG was available in 82% of these patients.

There was a significant 24-hour variation in onset of STEMI symptoms with peak onset at 09:00 (Figure 1), as estimated by Rayleigh test (p<0.001).

Baseline characteristics are shown according to the 6-hour intervals as described in the methods section. The characteristics were comparable between the different time groups except for age, gender, body mass index, heart rate, family history of heart diseases, and smoking status (Table 1).

There was a significant difference between the groups in pre-hospital delay,

Table 1. Baseline characteristics

Variable	00:00 - 5:59	6:00 - 11:59	12:00 - 17:59	18:00 - 23:59	P-value
N =	1269	2335	1998	1368	
Demographics					
Age (years)	62.8±12.6	64.0±12.6	63.2±12.6	62.2±12.9	<0.001
Sex (female)	30.1 (383)	26.9 (628)	25.9 (518)	28.4 (388)	0.046
Body mass index (kg/m2)	26.4 [24.4-29.3]	26.3 [24.2-28.9]	26.2 [24.2-29.0]	26.6 [24.5-29.4]	0.022
Heart rate	75.1±18.0	74.8±17.6	76.4±18.8	78.3±19.8	<0.001
Systolic blood pressure	130.5±26.1	129.7±25.7	129.1±26.3	130.8±27.3	0.288
Diastolic blood pressure	77.2±16.1	77.3±15.8	76.4±15.8	77.9±16.8	0.090
Heart rate >100 bpm	8.0 (91)	6.6 (137)	8.8 (158)	11.3 (137)	<0.001
Systolic blood pressure <90 mmHg	3.4 (39)	4.3 (90)	4.8 (86)	5.7 (69)	0.060
Medical history					
History of hypertension	39.1 (480)	34.9 (786)	35.5 (681)	35.5 (463)	0.085
History of diabetes	12.8 (161)	11.5 (265)	10.1 (198)	11.3 (154)	0.120
Hypercholesterolemia	25.2 (293)	24.6 (533)	22.5 (403)	24.1 (302)	0.288
Smoker	49.0 (592)	41.0 (900)	46.3 (867)	48.8 (634)	<0.001
Family history	46.2 (548)	40.9 (895)	41.4 (777)	43.1 (553)	0.021
History of MI	9.2 (115)	9.3 (214)	9.6 (187)	8.8 (119)	0.913
History of PCI	7.5 (95)	8.7 (201)	8.6 (172)	7.7 (105)	0.502
History of CABG	3.1 (38)	3.1 (72)	2.7 (53)	2.1 (28)	0.255
Time variables					
Ischemic time (minutes)	224 [160-370]	197 [147-282]	177 [132-250]	180 [135-255]	<0.001
Pre hospital delay (minutes)	173 [110-300]	150 [102-238]	130 [90-195]	137 [95-210]	<0.001
Door to balloon (minutes)	40 [27-61]	43 [27-65]	40 [27-61]	36 [26-53]	<0.001
Laboratory values					
CK maximum (mg/L)	1540 [616-3268]	1300 [531-2886]	1285 [568-2750]	1305 [571-2923]	0.004
CKMB maximum (mg/L)	197 [84-360]	170 [73-322]	162 [76-311]	171 [77-315]	0.002
Angiographic characteristics					
Anterior infarction	46.6 (585)	43.7 (1004)	41.2 (810)	42.6 (576)	0.021
1 vessel disease	43.7 (551)	46.5 (1073)	46.0 (910)	47.1 (639)	0.320
Stent placement	86.9 (1099)	86.8 (2006)	87.1 (1725)	86.9 (1180)	0.995
Thrombus aspiration	37.2 (467)	37.5 (862)	39.3 (774)	37.7 (509)	0.569
TIMI flow pre-PCI					0.025
0	58.5 (734)	54.1 (1247)	55.1 (1089)	51.1 (693)	
1	8.4 (105)	9.7 (224)	9.4 (186)	8.9 (121)	
2	16.0 (201)	16.0 (369)	16.5 (327)	18.3 (248)	
3	17.1 (215)	20.1 (464)	19.0 (375)	21.6 (293)	
TIMI flow post-PCI					0.322
0	0.8 (10)	1.4 (33)	1.5 (29)	0.8 (11)	
1	1.2 (15)	1.6 (37)	1.0 (20)	1.0 (14)	
2	9.4 (118)	8.7 (201)	9.4 (186)	9.6 (130)	
3	88.6 (1111)	88.2 (2032)	88.1 (1738)	88.6 (1201)	
Myocardial Blush Grade					0.036
0	4.2 (44)	5.3 (99)	5.1 (83)	5.4 (63)	
1	19.7 (206)	16.1 (299)	15.0 (244)	14.4 (169)	
2	35.3 (369)	37.0 (685)	37.5 (611)	35.4 (415)	
3	40.7 (425)	41.6 (770)	42.5 (692)	44.8 (526)	
Myocardial Blush Grade 2/3	62.6 (794)	62.3 (1455)	65.2 (1303)	68.8 (941)	0.058
Successful PCI	73.6 (768)	75.9 (1406)	77.4 (1261)	77.8 (913)	0.067
Outcomes					
30 day mortality	2.8 (36)	3.9 (92)	4.5 (90)	5.6 (76)	0.005
1 year MACE	14.7 (187)	16.3 (381)	15.8 (317)	16.3 (222)	0.637
1 year mortality	5.2 (66)	6.9 (161)	7.0 (140)	7.4 (101)	0.111

The data are mean±SD, median [IQR] or percentages (numbers). Bpm = beats per minute; CABG = coronary artery bypass graft; CK = creatine kinase; CKMB = myocardial band of creatine kinase; IQR = interquartile range; LAD = left anterior descending artery; LCx = left circumflex artery; MACE = major adverse cardiovascular event; MI = myocardial infarction; mmHg = millimeter of mercury; PCI = percutaneous coronary intervention; RCA = right coronary artery; SD = standard deviation; TIMI = Thrombolysis In Myocardial Infarction.

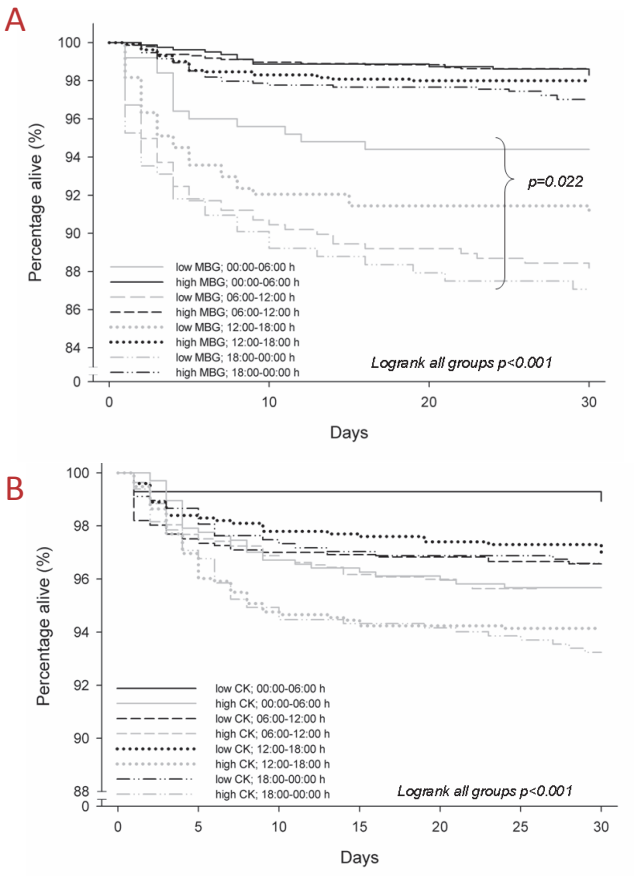
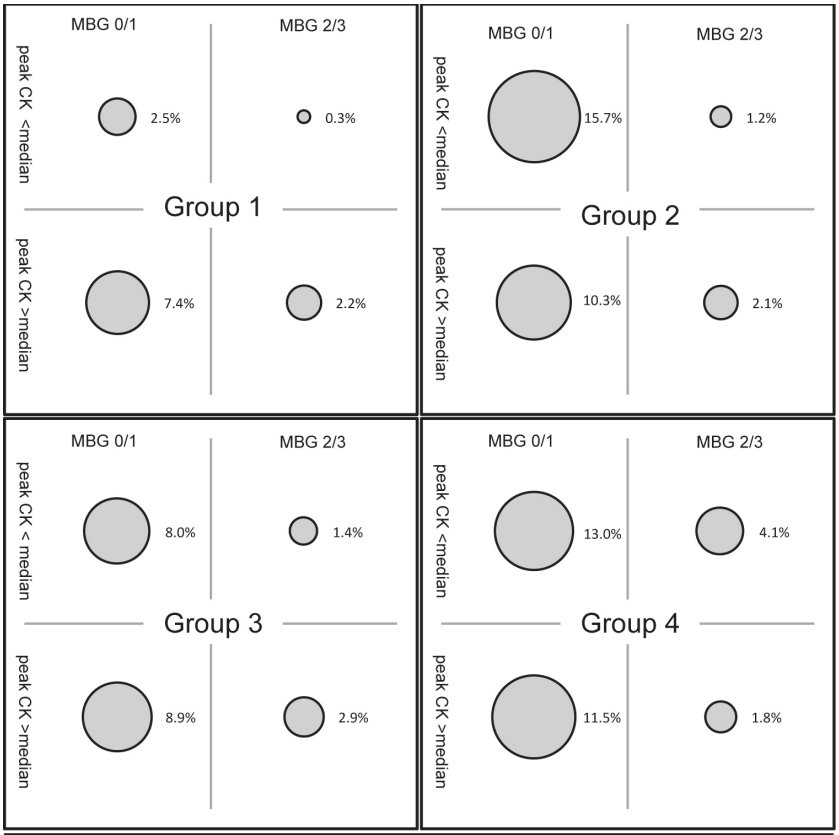


Figure 2(a). Thirty-day survival rates of high and low MBG per group - Thirty-day survival rates of MBG (MBG 2/3) and low MBG (MBG 0/1) per group of symptom onset time. (b) **Thirty-day survival rates of above and below median CK levels per group -** Thirty-day survival rates of low CK (below median CK) and high CK (above median CK) levels per group of symptom onset time. h=hour.

ischemic time and door-to-balloon times. Most strikingly, patients in group 1 encountered longer pre-hospital delay and consequently also longer ischemic time. Angiographic characteristics were comparable between the groups except for anterior infarction and TIMI flow pre-PCI. Procedural success was not different between the groups. Achievement of MBG 2/3 was not significantly different between the groups, although there was a trend toward higher grades in group 4. MI size differed significantly between the groups, with highest median CK and CKMB values in group 1.

Follow-up was available for all patients. The 30-day mortality rate was significantly different between the groups, with lowest in the group 1. However, in multivariable Cox regression analysis time of symptom onset, modeled with four degrees of freedom function, was neither associated with 30-day mortality nor 1-year mortality (Table 2). Both MI size and MBG were independent predictors of 30-day and 1-year mortality. Survival analyses were performed for each group categorized into low MBG (MBG 0/1) and high MBG (MBG 2/3), and for each group categorized into below and above median CK level. The categories with high MBG had all a favorable survival,



Group		OR (95% CI)	p-value
1	MBG0/1 CK<median	9.584 (0.858 – 107.031)	0.996
	MBG0/1 CK>median	29.512 (3.805 – 229.034)	0.018
	MBG2/3 CK<median	*Reference*	
	MBG2/3 CK>median	8.408 (1.060 – 66.685)	0.658
2	MBG0/1 CK<median	68.575 (9.123 – 515.457)	0.001
	MBG0/1 CK>median	42.451 (5.721 – 314.797)	0.004
	MBG2/3 CK<median	2.936 (0.352 – 24.279)	1.000
	MBG2/3 CK>median	7.875 (1.031 – 60.127)	0.699
3	MBG0/1 CK<median	32.243 (4.038 – 257.440)	0.016
	MBG0/1 CK>median	36.098 (4.782 – 272.505)	0.008
	MBG2/3 CK<median	5.356 (0.683 – 41.999)	1.000
	MBG2/3 CK>median	11.083 (1.469 – 83.635)	0.295
4	MBG0/1 CK<median	48.130 (6.001 – 385.997)	0.004
	MBG0/1 CK>median	55.205 (7.316 – 416.552)	0.002
	MBG2/3 CK<median	6.682 (0.843 – 52.974)	1.000
	MBG2/3 CK>median	15.921 (2.108 – 120.234)	0.109

Figure 3. Bubble-plot of percentage 30-day mortality per group categorized by myocardial blush grade (0/1 vs. 2/3) and CK levels (below vs. above median) - 30-day mortality is presented per category of blush (0/1 vs. 2/3) and peak CK levels (below vs. above median) for each time of symptom onset group. The size of the bubble presents the percentage of deaths per category, relative to the largest percentage of all categories. The accompanying table shows logistic regression analysis of all groups, with the group with lowest mortality serving as a reference category. P-values are corrected for multiple testing, using bonferroni correction.

Table 2. Cox-regression analysis of 30-day and 1-year mortality

	30-day mortality		1-year mortality	
	HR (95% CI)	P- value	HR (95% CI)	P- value
Time of symptom onset*	..	0.7303	..	0.8169
Age	1.06 (1.05 - 1.08)	<0.001	1.07 (1.06 - 1.08)	<0.001
Gender	0.90 (0.63 - 1.28)	0.551	0.96 (0.74 - 1.24)	0.733
History of diabetes	1.24 (0.82 - 1.88)	0.307	1.70 (1.27 - 2.27)	<0.001
Systolic blood pressure <90 mmHG	8.27 (5.85- 11.70)	<0.001	5.51 (4.07 - 7.47)	<0.001
Heart rate >100 bpm	3.39 (2.39 - 4.81)	<0.001	2.91 (2.19 - 3.86)	<0.001
Peak CK	1.45 (1.07 - 1.95)	0.015	1.29 (1.03 - 1.61)	0.029
Ischemic time	1.06 (0.70 - 1.60)	0.796	1.12 (0.81 - 1.58)	0.479
MBG	0.54 (0.45 - 0.63)	<0.001	0.62 (0.55 - 0.71)	<0.001

* Four degrees of freedom function. P-value of likelihood ratio test is given.
Bpm = beats per minute; CK = creatine kinase; MBG = myocardial blush grade; mmHg = millimeter of mercury.

which did not differ between the groups according to the time of day. However, there was a significant difference in the survival of the groups categorized into low MBG. Patients presenting with start of symptoms between 00:00 and 06:00 (group 1) had a significantly improved outcome when compared to the patients within the other groups (logrank: $p<0.022$; Figure 2a). When categorized according to below or above median CK levels, survival was significantly different between the groups with favorable survival in patients with symptom onset time 00:00 – 06:00 and below median CK levels (logrank $p<0.001$; Figure 2b). To gain further insight into the relationship of MBG and MI size on 30-day mortality during the day, these variables were plotted in a bubble-plot for each group (Figure 3). The percentage of deaths per category (based on high/low MBG and above/below median CK levels) showed a distinct pattern of distribution between the groups. Specifically, favorable outcomes were noted in group 1 patients presenting with low MBG and below median CK levels.

Discussion

The findings of this study can be summarized as follows: (1) we confirm in our combined registry of STEMI patients that there is a significant 24-hour pattern in the onset of symptoms, with a peak of onset at 09:00 hour; (2) patients with onset of symptoms between 00:00 and 06:00 hour have a longer ischemic times and accordingly larger enzymatic MI sizes; (3) procedural success is comparable throughout 24-hours; (4) despite the longer ischemic time and bigger MI size, this did not translate into higher 30-day and 1-year mortality in patients with onset between 00:00 and06:00 hour. In contrast, mortality was lowest in this group, which is an unexpected finding; and (5) patients with low MBG presenting in the early morning hours have better prognosis,

suggesting a 24-hour pattern in relation of MBG and MI size with outcome.

Several previous studies described a circadian variation in the incidence of acute MI and sudden cardiac death. A peak with onset of symptoms in the morning between 06:00 and noon is well established [10,12,19,23,24,25]. A meta-analysis containing 30 studies showed that 31.6% of acute MI onset is observed in this morning period [26]. The circadian rhythm of symptom onset has been ascribed to diurnal variations in autonomic nervous activity, fluctuations in platelet and coagulation activity, and catecholamine levels [27,28,29,30]. It has been reported that pharmacological treatment alters circadian rhythm, especially beta-blockers, aspirin and calcium blockers [3,5,30,31]. Additionally, the schedule of ingestion of medication may influence a patients risk for cardiovascular events. Unfortunately, we lacked data on maintenance medication in our cohort.

In our study, ischemic time was significantly different between the groups with longest ischemic times in the 00:00 – 06:00 hour group (p<0.001). The direct transport to a catheterization laboratory for primary PCI, and thereby circumventing other hospitals or the emergency departments, reduces time from the first medical contact to coronary intervention [32]. With a standardization of this protocol similar response and transportation times can be reached 24 hours a day. Unfortunately we lack data on the times between the STEMI diagnosis and arrival of the ambulance at the hospital. However, prehospital triage and transport to the PCI center is uniform around the clock, and thus we can assume that the longer ischemic times in the 00:00 – 06:00 hour group are mainly due to longer patient related delay. Patients with symptom onset between 00:00 and 06:00 hour wait longer before they contact medical services. This is in accordance with the results of Holmes et al. [19]. To some extent the prehospital delay during the night could be affected by slower response times of emergency medical services personal. Indeed in a retrospective study analyzing 568 calls for medical help because of out-of-hospital cardiac arrest it was observed that the response times were slowest during the night and fastest during the afternoon [33]. However, door-to-balloon times and angiographical success of the primary PCI did not differ according to the time of day in our study, suggesting stable performance of healthcare professionals.

It has been questioned whether the quality of treatment with PCI is equal during normal duty hours and off duty hours. Several studies have assessed in-hospital, medium and long-term outcomes for PCI therapy during on-hours compared to off-hours. Distinct longer delay times during off-hours are found in most of these studies, but there are conflicting results in terms of angiographic and clinical outcomes for patients treated during off-hours [34,35,36,37,38,39,40,41,42,43,44,45]. These conflicting results may be due to presentation of sicker patients and less successful PCI procedures during

off-hours [46]. However, a recent study by Noman et al. did not find any differences [40]. In our study, procedural success rates showed no significant differences between the groups, although there was a trend towards lower success rates in group 1.

A circadian pattern is also seen in the effectiveness of treatment of acute STEMI. Although primary PCI is a more effective strategy in STEMI patients than thrombolytic therapy [47], both treatment methods show circadian patterns in effectiveness. Thrombolytic therapy shows lower reperfusion rates in the early morning hours probably due to circadian fluctuation of endogenous hemostasis and thrombolytic activity [48,49]. Intrinsic mechanisms may influence myocardial perfusion after primary PCI as well. MBG was significantly lower in patients undergoing primary PCI during the night hours (00:00-08:00) (38). Recently it was observed that in STEMI patients treated with primary PCI epicardial and microvascular patency was more impaired when infarction started in the period between 18:00 and 05:59 hour. Corrected TIMI frame count and ST-segment resolution were used as measures of epicardial and microvascular patency (50). In our study there was no significant difference in the achievement of post PCI TIMI flow 3 and MBG 2/3 between groups, although there was a trend towards a higher proportion of MBG 2/3 in group 4. When analyzed in the two time groups according to Suzuki et al. there was no significant difference either (data not shown).

In our study, infarct size was significantly larger in the 00:00 – 06:00 hour group. This may be attributed to a longer ischemic time and a higher incidence of anterior MI in this group [51]. However, other factors may potentially also play important roles in the circadian variation of infarct sizes [11,12]. Durgan et al. showed in a mice model that there is an association between time of day and tolerance of the heart to ischemia/reperfusion, resulting in larger infarct size injury at the sleep-wake transition, and reduced left ventricular function 1 month later [52]. The time of day variation was lost when the mice were genetically ablated for the cardiomyocyte circadian clock. When translated to humans, MI size and left ventricular function were found to be dependent on time of occlusion of the epicardial vessel, suggesting a time-dependent variation in the tolerance to ischemia/reperfusion [11,12]. However, these studies were small and monocentric and a larger multicenter analysis could not confirm these findings [53].

In our study population, the crude 30-day mortality was lower in group 1, which seems contradictory, especially since this group is associated with large MI sizes and therefore one would expect impaired prognosis. Importantly, this finding is consistent when analyzed separately per hospital (data not shown). Nevertheless, when adjusted for other parameters, the group variable was not associated with mortality.

Interestingly, mortality was lowest in group 1 patients with low MBG compared to the other time groups. These patients might represent a group of patients who have a higher tolerance for ischemia compared to others.

Of note, obstructive sleep apnea is associated with an increased incidence of acute MI between 00:00 and 12:00 hour [54,55]. Hypoxic periods are common in sleep apnea patients, which may result in a better tolerance to myocardial ischemia.

Recent other studies showed no differences in outcomes in terms of in-hospital mortality between different time groups [12,19]. However, Fournier et al. reported a significant higher mortality at 30-days in patients with symptom onset between 00:00 and 06:00 hour compared to the rest of the 24-hour period [10].

As shown in Figure 3, the percentages of deaths per MBG and peak CK category are different amongst the groups and therefore the association of MBG and peak CK levels with 30-day mortality might be different during the day. This is an interesting finding that warrants further study into the relationship of CK, as a reflection of myocardial injury, with MBG, as a reflection of microvascular perfusion after mechanical treatment, on outcome.

Several aspects have to be taken into account when interpreting our results in light of other articles. The inclusion criteria between the studies are somewhat different: Reiter et al. included only patients with ischemic times <6 hours and TIMI flow grade 0 before PCI, whereas Suarez-Barrientos et al. excluded all patients with previous MI [11,12]. On the other hand, Ammirati et al. analyzed their cohort applying the exclusion criteria of both aforementioned studies [53].

Survivor bias, meaning that in our study less high-risk patients were reaching the hospital before deceasing during the 00:00–06:00 hour period, may be of importance for our findings. From studies in cardiac arrest patients, it is known that return of spontaneous circulation and survival are lower in in-hospital and out-of-hospital cardiac arrest patients during night hours [56,57]. In addition, 30-day survival rates are significantly lower in patients with out-of-hospital cardiac arrest at night [57].

This analysis has several limitations. First, the parameter time of symptom onset relies on patient's retrospection and is therefore susceptible to recall bias. Second, no information was available on the patient chronotype nor on employment on night or shift work schedules, which is suggested to influence the clock time of acute MI onset [58]. Third, only patients that presented to the hospital and received primary PCI were included in the analysis; patients not reaching the hospital alive or not receiving primary PCI were therefore not included and could have been a confounder to our results. Fourth, information on medical treatment as well as time of ingestion of medication is lacking in our data; this may be of influence on circadian rhythms and

as such to our findings.

Conclusions

STEMI patients have a significant 24-hour pattern in onset of symptoms, with peak onset at 09:00 hour. MI size and MBG but not time of symptom onset are associated with 30-day and 1-year all cause mortality. The relation of MBG and MI-size with mortality differs throughout the day. Patients with failed reperfusion presenting in the early morning hours have better prognosis, suggesting a 24-hour pattern in relation of myocardial perfusion and infarct size with outcome.

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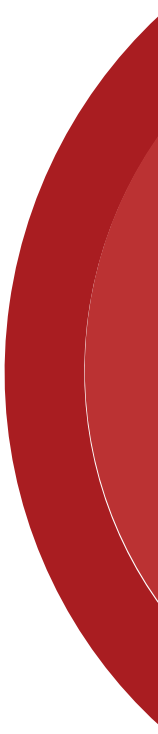

Clinical advances in imaging: how useful is computed tomography for guiding and evaluating cardiac interventions

4

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Summary

Multislice computed tomography (MSCT) has emerged as a non-invasive imaging modality allowing anatomical imaging of the heart. The presence of coronary artery stenosis can be reliably ruled out without development of cardiovascular events on follow-up, which currently makes MSCT particularly useful for evaluation of patients with chest pain and low to intermediate pre-test likelihood of coronary artery disease. In addition, MSCT may be useful in guiding interventions of cardiac valves or treating cardiac rhythm disorders. Since the technology of MSCT continues to evolve at a rapid pace and the radiation doses decrease, further expansion of the applications of MSCT across the clinical practices is expected.

Introduction

Following its introduction in the late 1990s, during the subsequent decade multi-slice computed tomography (MSCT) has rapidly evolved as a modality allowing imaging of the heart. Three-dimensional imaging of the entire heart during a single breath hold is currently possible. In particular, the potential of MSCT to visualize coronary artery lumen and wall in a non-invasive manner gained tremendous interest. Indeed, MSCT coronary angiography may be performed to assess coronary artery stenosis in symptomatic patients with suspected coronary artery disease (CAD) [1,2]. Moreover, MSCT coronary angiography may potentially be useful in guiding coronary interventions and in evaluation of the results of treatment [1,2]. In addition, three-dimensional anatomical information obtained during the examination may be clinically useful in guiding interventions of the cardiac valves or treating rhythm disorders [1,2].

In this review we provide an overview of the current applications of MSCT, discussing the areas in which MSCT may replace invasive imaging and areas in which MSCT may be useful in guiding cardiac interventions.

Principles of imaging of the heart with multi-slice computed tomography, safety issues

MSCT scanners consist of an x-ray source and detectors mounted on opposite sides of a gantry that continuously rotates around the patient. The scans are performed as the patient moves through the gantry. Computer systems can process these data to generate three-dimensional volumetric information, which is in turn viewable from multiple different projections on workstation monitors.

The most important parameters related to computed tomography (CT) image quality are the ability to depict differences between tissues, i.e. spatial resolution, as well as temporal resolution and volume coverage during a single gantry rotation. The temporal resolution is essentially determined by the rotation speed of the gantry, the number of x-ray sources (single versus dual source MSCT), and the number of the heart cycles used in a reconstruction. Indeed, the development of dual source MSCT, incorporating two x-ray tubes mounted at approximately 90 degrees angle, allows obtaining images during one fourth of gantry rotation. Since the introduction of MSCT the number of detector rows has grown from 4 to as far as 320, allowing the coverage of 16 centimeters in a single gantry rotation. A reduction in slice thickness, increase in gantry rotation speed, and increased number of detector rows were paralleled by a significantly improved image quality. With a spatial and temporal resolution of 0.4-0.6 mm and 83-175 ms, respectively, 64-slice MSCT scanners are currently considered a minimum prerequisite for non-invasive imaging of coronary arteries [3].

The beating heart can be scanned in two ways [4]. The first method is electrocardiogram (ECG)-triggered prospective axial sequential scanning whereby acquisition is triggered by the ECG signal in a pre-defined phase of the cardiac cycle. After individual axial image acquisition the table moves (along the z-axis) to the next position for the next scan, which is repeated until the entire heart is scanned. The second method involves scanning with an application of an ECG-gated retrospective spiral mode with continuous data acquisition while the patient moves through the gantry until the entire heart is covered.

Currently, invasive coronary angiography is a gold standard to detect CAD. However, MSCT may serve as an alternative in certain patient populations, particularly due to its non-invasive approach (associated with a lower risk of procedural complications) and lower costs. Nevertheless, it is important to realize that the spatial and temporal resolution of invasive angiogram is superior as compared to MSCT (0.2 mm and 20 ms, respectively). Accordingly, in order to acquire accurate results it is important to understand which factors may influence the image quality of MSCT and how the drawbacks of MSCT may be overcome. This is particularly important for the visualization of the small, fast moving coronary arteries. A regular cardiac heart rhythm and a slow heart rate are prerequisites to achieve an appropriate image quality. A heart rate below 65 beats per minute (and even below 60 beats per minute with the new flash CT systems) is required, which is often achieved with the administration of beta-blockers. Moreover, MSCT may be rejected in obese patients as diagnostic image quality may be unacceptable due to high image noise. In addition, extensive calcifications may negatively influence the diagnostic accuracy of MSCT coronary angiography by increasing the chance of false positive findings.

The most relevant disadvantages of MSCT are the necessity of iodinated intravenous contrast agent and radiation exposure [5]. Iodinated contrast agents may cause allergic reactions in a minority of patients (<1%) and are potentially associated with the development of renal dysfunction. Indeed, the administration of contrast exhibits a risk of significant contrast induced nephropathy, although the risk is low even in patients with moderate-to severe renal insufficiency [6]. Radiation exposure is of considerable concern with the growing use of imaging modalities involving ionizing radiation, since it may be associated with the development of malignancies. Consequently, enormous attention has been involved to dose reduction measures. First, avoiding unnecessary MSCT studies is the most powerful strategy in reducing radiation exposure and MSCT should be used according to the appropriateness criteria [1]. Second, tube voltage determines the radiation exposure and adjusting the voltage depending on patient size is feasible. The most frequently applied tube voltage is 120 kV, however adequate

image quality may be achieved using 100 kV in non-obese adults [5,7,8]. Even further reduction of tube current to 80 kV may be feasible in patients with a body mass index ≤ 22.5 kg/m^2 [9]. Moreover, distinctive scan modes may have an important impact on radiation dose. Indeed, prospective ECG triggered scanning may significantly reduce radiation exposure. Bischoff et al. observed a significant reduction of radiation dose, while maintaining image quality, when applying prospective as compared to helical scanning mode (3.6 mSv versus 11.2 mSv, p<0.001) [4]. Additionally, scan modes that cover the entire heart during a single heartbeat considerably reduce radiation dose. Novel 256- and 320-row CT systems allowing up to16 cm z-axis coverage allow scanning of the entire heart during a single gantry rotation. Without loss of image quality, effective radiation doses of 1.7-2.1 mSv may be achieved [10,11] High table speed through the gantry may be applied with the newest dual-source MSCT systems. These new generation MSCT systems have an increased number of detectors and faster gantry rotation speed, allowing a temporal resolution of as low as 75 ms. Using spiral scanning mode, with a high table speed, the entire heart may be scanned within one cardiac cycle allowing the radiation exposure in selected patients even below 1 mSv [12]. Importantly, those values are lower than the values obtained with conventional ICA, which range between 2 mSv to 15.8 mSv [13,14]

The areas in which multi-slice computed tomography can be superior to invasive imaging

Evaluation of coronary artery stenosis

Currently conventional ICA is considered the gold standard for the detection of coronary artery stenosis. Nevertheless, non-invasive MSCT has been recently proved to be a good alternative to ICA in certain patient populations. Several meta-analyses have been performed that evaluated the diagnostic performance of 64-slice MSCT and more recent scanners, demonstrating high per-patient sensitivity and specificity ranging from 97-100% and 82-91%, respectively [15-20]. Nevertheless, the most studies were retrospective analyses in small samples of patients with high pre-test likelihood of CAD, whereas the results were influenced by numerous biases. Accordingly, multi-center studies were performed to overcome the above limitations. In the recent multi-center trials aiming at identifying patients with coronary artery stenosis with 64-slice MSCT among individuals at low to intermediate pre-test likelihood for CAD and quantitative ICA serving as a standard of reference, MSCT was reported to have a sensitivity of 95-99% and a specificity of 64-83%, whereas a negative predictive value (NPV) was 97-99% (Table 1) [21,22]. In another recent multi-center multivendor trial, the sensitivity, and NPV of 64-slice MSCT angiography for detecting obstructive

CAD were 85% and 83%, respectively (Table 1) [23]. Based on those data it may be suggested that the diagnostic accuracy of MSCT is strongly influenced by the pre-test likelihood of CAD, as also recently demonstrated by Meijboom et al. Indeed, in patients with varying degrees of pre-test likelihood of CAD the sensitivity, positive predictive value (PPV) and NPV were 100%, 75% and 100% for low, 84%, 80% and 100% for intermediate and 98%, 93% and 89% for high pre-test likelihood of CAD, respectively [24]. Accordingly, MSCT angiography may be particularly suitable to exclude CAD in patients with low to intermediate pre-test likelihood of CAD (Figure 1). As a result, with the application of MSCT coronary angiography in clinical practice the number of diagnostic catheterisations associated with high costs and a certain risk of complications may be substantially decreased. MSCT coronary angiography may also be considered appropriate for the evaluation of patients who are unable to perform an exercise test or patients with equivocal test results. In a recent study including patients with inconclusive exercise tests, MSCT coronary angiography resulted in major cost savings, whereas the rate of invasive coronary angiography was reduced with no occurrence of adverse events [25]. Finally, the modality may also become an alternative to invasive coronary angiography in patients requiring coronary evaluation before non-coronary cardiac surgery or in patients with a new onset of heart failure of unknown aetiology [1].

The advantage of MSCT coronary angiography to rule out CAD is supported by prognostic studies performed in patients with suspected CAD. An important finding in these studies is that a normal MSCT coronary angiogram indicates an excellent prognosis. Indeed, in the meta-analyses including 7,335 and 9,592 patients with a median follow-up of 26.4 and 20 months the annualized event rate (including death, myocardial infarction, and revascularization) in patients with a normal MSCT coronary

Table 1. Diagnostic accuracy of 64-slice MSCT angiography in detection of significant coronary artery disease; multicenter trials, patient based analysis.

Author	No. of patients	Prevalence of CAD (%)	Sensitivity (%, 95% CI)	Specificity (%, 95% CI)	PPV (%, 95% CI)	NPV (%, 95% CI)
Budoff *et al* [9]	230	25	95 (85–99)	83 (76–88)	64 (53–75)	99 (96-100)
Miller *et al* [13]	291	56	85 (79–90)	90 (83–94)	91 (86-95)	83 (75–89)
Meijboom *et al* [10]	360	68	99 (98-100)	64 (53–73)	86 (53–73)	97 (94-100)

CAD: Coronary artery disease; CI: confidence interval; NPV: negative predictive value; PPV: positive predictive value.

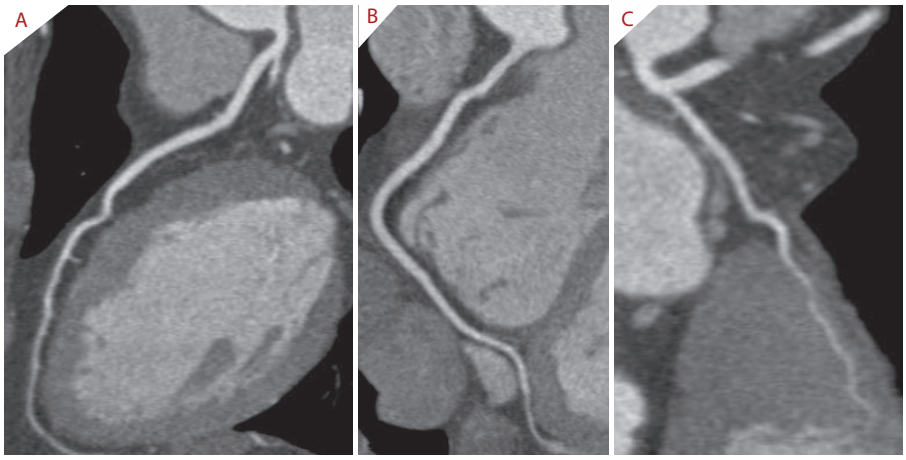

Figure 1. Example of multi-slice computed tomography angiography used to diagnose coronary artery disease. A 64-year-old woman presented to the outpatient clinic for evaluation of atypical chest pain in combination with a positive family history for coronary artery disease. Risk stratification resulted in an intermediate pre-test likelihood for coronary artery disease. Bicycle exercise test was inconclusive and subsequently a multi-slice computed tomography angiography was performed. Curved multiplanar reconstructions of the left anterior descending artery (A), the right coronary artery (B) and the left circumflex coronary artery (C) revealed normal coronary arteries.

angiogram was as low as 1.1% [26] and 0.17%, respectively [27]. On the other hand, the presence of obstructive lesions is clearly associated with a worse prognosis. The presence of ≥1 significant coronary artery stenosis was associated with a 10-fold higher risk for cardiovascular events [26]. Moreover, the risk of cardiovascular events may be increased with increasing severity of CAD.

Clinical implications of evaluation of coronary artery stenosis in patients with stable coronary artery disease

The recent publication of the results of multi-center trials evaluating the outcomes in patients undergoing medical therapy as compared to revascularization in non-diabetic and diabetic patients presenting with stable CAD triggered a discussion on the role of medical therapy in the above patient population. In the Clinical Outcomes Utilizing Revascularization and Aggressive Drug Evaluation (COURAGE) trial, 2287 patients with stable significant CAD and myocardial ischemia were included and were randomized for an optimal medical therapy alone or in combination with percutaneous coronary intervention (PCI). Surprisingly, at four and a half years PCI did not reduce the risk of death, myocardial infarction, or other cardiovascular events [28]. Similarly, in the Bypass Angioplasty Revascularization Investigation 2 Diabetes (BARI 2D) trial, 2368 with type 2 diabetes and stable CAD were stratified to undergo either prompt revascularization

along with medical therapy or intensive medical therapy alone. At five years no significant difference was observed in the rates of death and major cardiovascular events between patients undergoing revascularization and optimal medical therapy alone [29]. Even if the above studies have important limitations, the results of the studies may have implications also for the application of MSCT in clinical practice. In the COURAGE and the BARI 2D trials, the patient inclusion was based on the findings on ICA. Accordingly, the data may not be generalized to patients with lower risk coronary anatomy who do not undergo an initial ICA. As the reported diagnostic accuracy of MSCT coronary angiography to detect obstructive CAD is high it is suggested that non-invasive MSCT may play a role in an initial evaluation of CAD in patients with stable angina in order to rule out three-vessel or left main disease. Indeed, based on the results of the COURAGE and BARI 2D trials, the need for intervention in patients with stable CAD may be decreased. Moreover, MSCT also allows detection of borderline obstructive or non-obstructive lesions, which may be considered for non-invasive management.

Nevertheless, several important issues need to be taken into consideration. First, due to limited spatial resolution of MSCT as compared to ICA a number of false positive findings may be observed on MSCT. Accordingly, an accurate assessment of severity of individual stenosis may be difficult in patients with extensive coronary artery calcifications, which is usually the case in patients with type 2 diabetes. Second, high diagnostic accuracies of MSCT angiography have been reported in the studies performed in the centers of excellence. Nevertheless, one should realize that the diagnostic accuracy of MSCT coronary angiography may be substantially lower in the real world when it is performed in the centers with limited experience in performing and assessing MSCT coronary angiograms [30]. Future studies are clearly necessary to investigate this approach.

Evaluation of patients with suspected acute coronary syndromes

The majority of studies evaluating the diagnostic accuracy of MSCT coronary angiography are performed in the patients presenting with chest pain not in the emergency setting. Nevertheless, another possible application of MSCT is the diagnosis/exclusion of CAD in the clinical setting of suspected acute coronary syndromes (ACS). Indeed, up to 8% of patients with ACS are misdiagnosed and inappropriately discharged home [31]. A number of studies were performed aiming to evaluate the effectiveness of MSCT in the early triage of patients with suspected ACS [32,33]. The ability of MSCT to exclude ACS after a normal MSCT scan or non-obstructive coronary lesions has been observed [33]. Moreover, in a study Goldstein et al compared

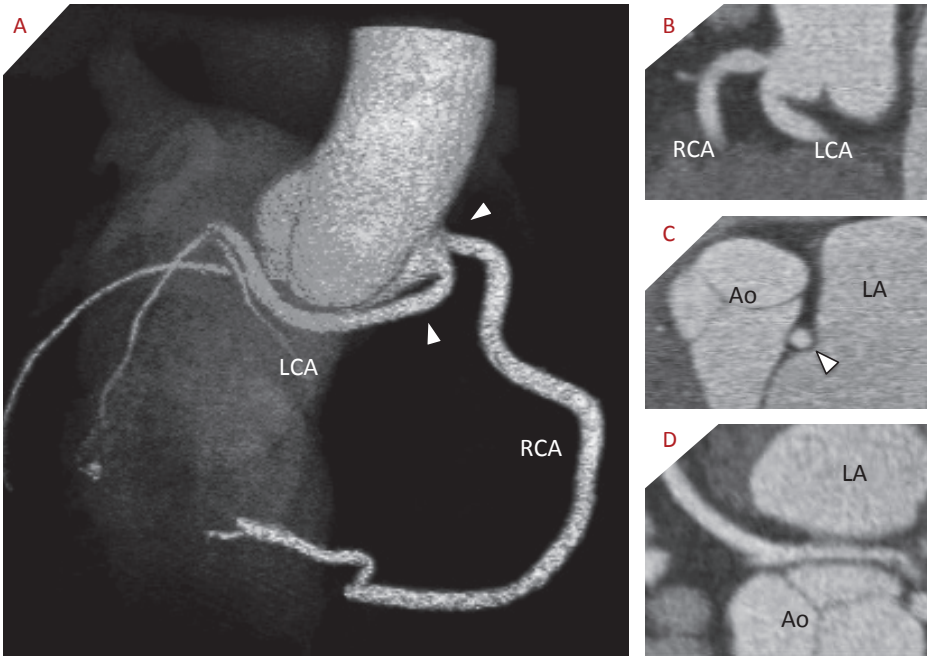

Figure 2. Left coronary artery, originating from the right coronary cusp with a retro-aortic course (between aorta and left atrium). (A) A 3D volume rendered reconstruction shows the LCA originating from the right coronary cusp and a retro-aortic course (arrowheads) towards the left side of the heart. (B) a maximum intensity projection image demonstrates the origin of the LCA from the right coronary cusp. (C & D) show the course of the LCA between the left atrium and aorta. This particular anomalous coronary artery is considered to be benign.
Ao: Aorta; LA: Left atrium; LCA: Left coronary artery; RCA: Right coronary artery.

two diagnostic strategies (standard of care versus MSCT coronary angiography), where the diagnostic efficiency, safety and cost-effectiveness of both strategies in patients presenting with low-risk ACS (no ECG changes, no elevation of cardiac enzymes) were evaluated [32]. The standard of care involved clinical observation as well as serial testing with ECG and cardiac enzymes, followed by a myocardial perfusion scan with single photon emission computed tomography (SPECT) for the evaluation of myocardial ischemia. The MSCT arm included initial MSCT, followed by ischemia testing with SPECT in case of intermediate coronary lesions or non-diagnostic MSCT scans. The median diagnostic work-up duration in the standard of care arm was 15 hours, whereas it was 3.4 hours ($p<0.001$) in the MSCT arm, resulting in significantly lower costs associated with MSCT arm ($1,872 versus $1,586 $p<0.001$). Nevertheless, this was at the expense of a double or triple radiation exposure (MSCT, followed by SPECT and in some cases by ICA) in 24% of patients in the MSCT arm. Summarizing the above results, the experts consider MSCT coronary angiography as an appropriate modality in the diagnosis/exclusion of CAD in patients with low-risk ACS [1,34]. Nevertheless,

as MSCT currently does not allow evaluation of hemodynamic significance of the lesions and because MSCT may be inconclusive, a proportion of patients with low probability of ACS may need additional testing. Moreover, performing MSCT coronary angiography in the setting of ACS may currently be logistically challenging in a lot of centers. Future studies are necessary to explore different diagnostic protocols in the early triage of patients with ACS.

Evaluation of coronary artery anomalies

In a recent study the prevalence of coronary anomalies with MSCT was 5.7% [35]. Coronary anomalies are reported to be correlated with sudden cardiac death, predominantly in young healthy individuals. Numerous studies have reported on the accuracy of MSCT to detect anomalous coronary arteries as compared to ICA. Indeed, in a study including 23 patients, the accuracy of MSCT to detect coronary artery anomalies was 100% [36]. In addition, in a series with 380 patients with coronary artery anomalies, 58 patients had MSCT performed after ICA. Among those 58 patients, MSCT allowed a better assessment of the anatomic course and origin of the coronary arteries in 8 (14%) cases, whereas ICA failed to identify them in 10 (17%) cases [37]. As ICA represents a two-dimensional lumenogram of the coronary arteries, a reliable interpretation of the course of anomalous coronary arteries may be difficult. MSCT providing three-dimensional information may be a more robust modality in identifying malignant coronary anomalies requiring treatment (Figure 2).

Although identification of anomalous coronary arteries with MSCT is possible, the clinical importance of the findings is less well established. Surgical correction of anomalous origin of coronary arteries is generally considered best for children and young adults. However, in a recent study no positive impact on long-term survival could be demonstrated in older adults when surgical correction of the anomalous course of the aberrant coronary artery was performed as compared to medical therapy [38]. The topic deserves further investigations.

The areas in which multi-slice computed tomography may assist in cardiac interventions

Evaluation of chronic total occlusion

Chronic total occlusions (CTO) are common in patients referred for ICA [39]. Successful recanalization of a CTO is associated with substantial improvement in symptoms, physical limitation and quality of life [40]. Nevertheless, recanalization of a CTO remains challenging because of the inability to visualize the vessel lumen if the occluded segment is long and tortuous. Accordingly, the success rates in treating CTO still range

between 55% and 85% [39].

Since MSCT allows visualization of not only coronary artery lumen but also composition of the vessel wall, it may provide complementary information that could be helpful in planning the recanalization procedure and thereby increase the procedural success. CTOs appear as regions of the coronary artery that are not filled with intraluminal contrast. The distal part of the occluded vessel is filled with contrast through collaterals and is less intensively opacified. Calcifications can be clearly visible as bright structures along the occluded vessel (Figure 3). The factors associated with failure of recanalization as identified with ICA are occlusion length of ≥20 mm, severe calcification, blunt stump, tortuosity of occluded vessel, presence of bridging collaterals, as well as side branch at occlusion site. Several rather small studies have attempted to investigate the value of MSCT in predicting the outcomes of recanalization of CTO [41-44]. The findings on MSCT associated with procedural failure were various parameters of lesion calcification, tortuosity or bending of coronary vessel, as well as shrinkage of the target vessel (Table 2). Accordingly, it seems that MSCT currently does not provide information complementary to ICA that might be useful in planning recanalization. Moreover, due to superior spatial resolution some parameters are even better visible on ICA (bridging collaterals or a side-branch at the entry point of occlusion). Keeping in mind still substantial radiation dose of current 64-slice MSCT systems (which is applied to patients who will subsequently undergo a

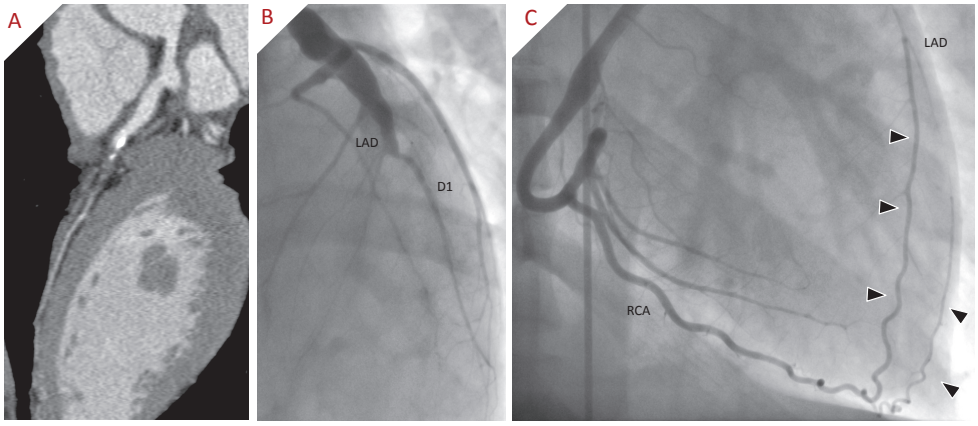

Figure 3. Computed tomography angiography in a 20-year-old male known with Kawasaki disease. Multislice computed tomography was performed for follow-up of known dilated coronary arteries. **(A)** multiplanar reconstruction of the LAD shows a dilated proximal part of the coronary artery with a totally occluded segment beyond a calcified plaque. **(B)** Distally the coronary artery is small in diameter. Invasive coronary angiography shows an aneurysmatically dilated LAD with a chronic total occlusion at the site where the first diagonal branch splits from the LAD. **(C)** Extensive collaterals (arrowheads) from the right coronary artery allow filling the distal LAD with contrast.
D1: First diagonal branch; LAD: Left anterior descending coronary artery; RCA: Right coronary artery.

Table 2. Predictors of success of treatment of chronic total occlusion as observed on MSCT coronary angiography.

Author	CT scanner type	No. of patients	No. of lesions	Parameters associated with procedural failure
Soon *et al* [24]	16-slice MSCT	39	43	Calcification >50% CSA
Garcia-Garcia *et al* [25]	16-slice MSCT 64-slice MSCT	139	142	Tortuosity of coronary vessel, calcification >50% CSA
Cho *et al* [26]	64-slice MSCT	64	72	Severe calcification
Ehara *et al* [27]	64-slice MSCT	110	110	Vessel bending, vessel shrinkage, severe calcification

CSA: cross sectional area; MSCT: multi-slice computed tomography

high radiation exposure recanalization procedure), the need to administer nephrotoxic contrast, and the lack of evidence that MSCT improves the outcomes, its application in planning of recanalization of CTO remains questionable. Additionally, MSCT may be useful in co-registration of images while performing recanalization procedures with magnetic navigation. Nevertheless, the value of MSCT in the above procedures needs to be demonstrated [45].

Evaluation of the aortic valve in transcatheter valve replacement

Transcatheter aortic valve implantation (TAVI) is a novel method to treat symptomatic severe aortic stenosis in patients with high surgical risk [46]. Although the results of the procedure are encouraging [47], several issues remain a concern. A mild aortic regurgitation is observed in 50% of patients, whereas 13-18% of patients develop a moderate regurgitation [46,48]. Moreover, vascular complications remain a safety concern. Accordingly, before the aortic valve implantation, extensive planning of the procedure is mandatory in order to increase the chance of success. Currently two devices are used, namely the self-expandable CoreValve revalving system (Medtronic, MN, USA) and the balloon expandable Edwards Sapien valve (Edwards Lifesciences, CA, USA). Proper device sizing and expansion as well as vascular access are of particular importance in the application of both devices.

The assessment of the aortic valve, aorta, and the peripheral vessels is usually performed using two-dimensional transthoracic and trans-oesophageal echocardiography, and angiography. Accurate measurement of the aortic annulus is one of the key steps in choosing a proper device. Indeed, undersized device may lead to suboptimal expansion and paravalvular regurgitation, whereas an oversized device may increase the risk of tissue rupture. However, the measurements with two-

dimensional echocardiography are limited since they are based on single annular plane assuming the aortic annulus is a circle. Nevertheless, using three-dimensional imaging modalities the annulus appeared to be a complex oval shaped structure (Figure 4) [49]. Indeed, Messika-Zeitoun et al. evaluated 45 patients referred for TAVI; despite good agreement of the sagittal diameter of the aortic annulus between transthoracic and transesophageal echocardiography (the mean difference between the measurements was 0.6±0.8 mm, p=NS), the difference between MSCT and transthoracic (1.22±1.3 mm, p=0.03) or transesophageal echocardiography (1.52±1.1 mm, p<0.001) was larger [50]. Moreover, Schultz et al. suggested that device selection could be more accurate if a mean diameter of an oval-shaped aortic annulus (a mean

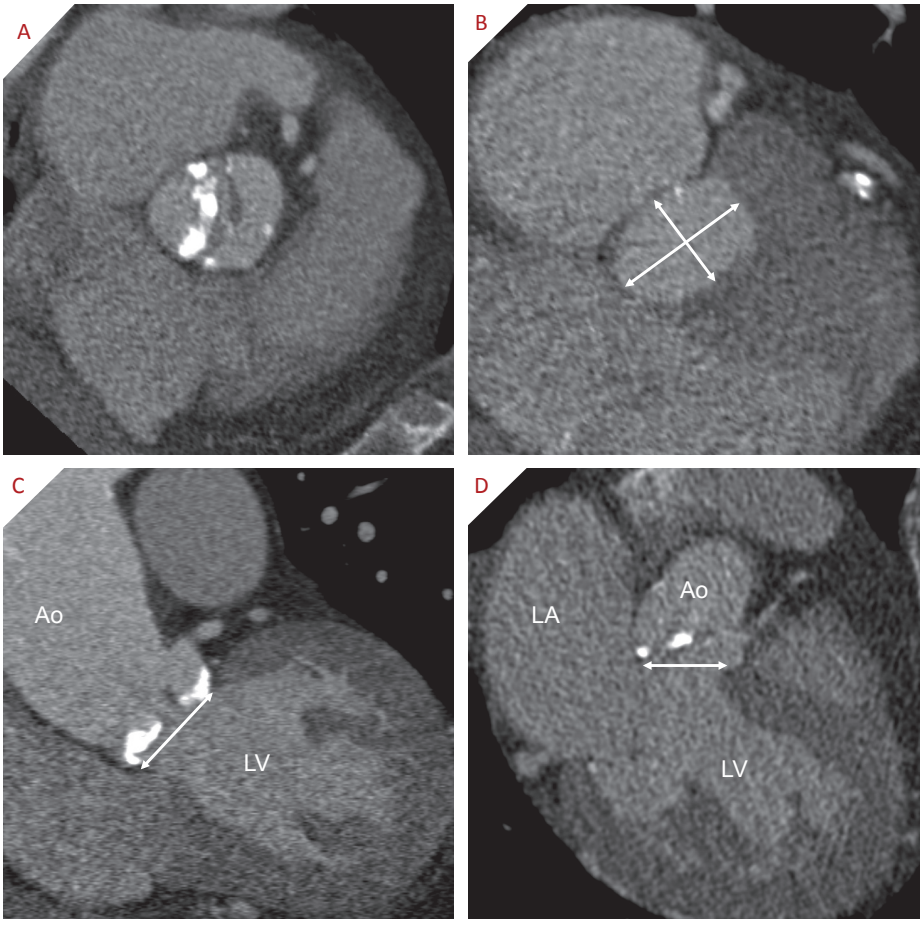

Figure 4. Assessment of the aortic valve annulus by multi-slice computed tomography. Oblique transverse view allows assessment of the degree of calcification of **(A)** the aortic valve (which in this case is functionally bicuspid), and **(B)** the oval shaped annulus size. The size of the aortic valve annulus is measured in the **(C)** coronal and **(D)**sagittal views, providing an estimation of the eccentricity of the aortic valve annulus.
Ao: Aorta; LA: Left atrium; LV: Left ventricle.

of sagittal and coronal diameters, as observed on three-dimensional examination) was used instead of making a decision based on a single diameter measurement (Figure 4) [51]. In addition, the measurement demonstrated a good reproducibility [52].

Besides measurement of the aortic annulus, three-dimensional non-invasive imaging by MSCT allows for assessment of extent and location of aortic valve calcification as well as evaluation of the geometry of the aortic root and left ventricular outflow tract (Figure 4) [49]. Aortic valve calcifications have been associated with the presence of post-procedural aortic regurgitation. Indeed, John and colleagues found a strong correlation ($r=0.86$, $p<0.001$) between severity of calcification and degree of post-procedural aortic regurgitation [53]. For appropriate positioning of the prosthesis, the prosthetic valve needs to be deployed perpendicular to the annulus of the native valve. Pre-procedural assessment of the aortic root in relation to the body axis with MSCT appears to be useful in prediction of the angle of implantation. Indeed, Kurra et al investigated the accuracy of MSCT aortography in predicting the angiographic planes used for TAVI procedures. No difference was observed between the cranial angulation in the LAO X-ray angiograms and MSCT images, whereas a small difference was observed between the caudal angulation in the RAO angiograms and matched MSCT.images [54]. Accordingly, as MSCT may accurately predict the procedural angiographic angulations, this may decrease the need for repeated contrast injections during the TAVI procedure and increase the accuracy of correct valve positioning. Gurvitch et al achieved excellent or satisfactory final prosthesis projection in 90% of cases in patients when MSCT was used for predicting angiographic deployment projections as compared to 65% of cases when MSCT was not used [55].

Moreover, MSCT allows assessment of the peripheral arteries and thoracic aorta and may help identify patients with unfavourable anatomy, such as small lumen diameter, tortuosity, and extensive atherosclerosis. Indeed, Kurra et al observed that 35% of patients referred for TAVI had unfavorable atherosclerotic iliofemoral disease, the majority of these patients having luminal narrowing of <8 mm in the iliofemoral arteries [56].

Finally, after TAVI, MSCT may be a valuable and complementary tool to evaluate the procedural results (deployment and location of the prosthesis) in order to understand the underlying mechanisms of post-procedural aortic regurgitation.

Currently, a novel modality to obtain three dimensional information in the cardiac interventional laboratory is emerging. The C-arm in the interventional laboratory can be used as a CT scanner. Accordingly, the same information, which is obtained on pre-procedural MSCT, can be obtained intra-operatively. This includes the characterization of the aortic valve calcification, the assessment of the relationship

between the coronary ostia and the aortic leaflets, the information necessary for the valve sizing and planned localization of the implant. Moreover, the application of the C-arm CT may enable the co-registration of the reconstructed image with the C-arm and fluoroscopy such that the image rotates with it during positioning, providing the operator an additional information to improve placement of the aortic valve [57].

Additional studies are necessary to establish a reference method for pre-procedural evaluation of the patients referred for TAVI and to demonstrate whether the risks associated with MSCT (radiation exposure and administration of nephrotoxic contrast) are justified by a better outcome (lower post-procedural aortic regurgitation rate and less vascular complications).

Evaluation of the mitral valve in percutaneous procedures

Despite recent advances in treatment strategies, surgery is denied in 49% of high-risk patients with severe mitral regurgitation [58]. Consequently, transcatheter- and miminally invasive surgical techniques have been developed as an alternative approach. Currently, clinical data are available on the use of a MitraClip device (Abbott Vascular, CA, USA) for leaflet repair, as well as on Carillon (Cardiac Dimensions Inc, WA, USA) and Monarc (Edwards Lifesciences, CA, USA) devices for coronary sinus annuloplasty. Accordingly, the broadening options for mitral valve repair have increased the demands on the reliability of morphologic assessment of the mitral valve. Echocardiography is currently the most widely used imaging modality in therapy decision-making. However, three-dimensional imaging modalities such as MSCT may be useful in the assessment of anatomy and geometry of the mitral valve. Indeed, MSCT could clearly have an additional value in the assessment of the coronary sinus and its relationship with the coronary arteries and the mitral valve annulus before percutaneous mitral valve annuloplasty. The data from a study by Tops et al. demonstrated the course of the circumflex coronary artery between the coronary sinus and the mitral valve annulus in as many as 68% of patients (which might result in acute ischemia during the procedure) [59]. Moreover, coronary sinus coursed superiorly to the mitral annulus in the majority of patients. The feasibility of a comprehensive mitral valve evaluation with MSCT was nicely demonstrated in a study by Delgado et al [60]. Additionally, Feuchtner et al. demonstrated in 112 patients a sensitivity of 96% and a specificity of 93% in diagnosing mitral valve prolapse with MSCT as compared with two-dimensional echocardiography [61]. Nevertheless, echocardiography remains the most important tool in evaluation of patients referred for mitral valve intervention,

whereas MSCT may be a reserve option in case the echocardiography fails to provide insight into the mechanisms of mitral valve disease.

Evaluation before catheter ablation of atrial fibrillation

In patients with atrial fibrillation who remain symptomatic under optimal medical therapy, catheter ablation is an effective treatment method [62]. MSCT may play a role in patient selection, during the catheter ablation procedure as well as in the evaluation of the procedural complications.

MSCT may be used in the assessment of the left atrial size as well as the presence of thrombus. Indeed, MSCT is a reliable method to measure the left atrial volume [63]. Nevertheless, due to radiation exposure, the need for contrast agent with MSCT and good results available with echocardiography it is not routinely performed for the assessment of the left atrial size. Moreover, thrombus in the left atrium or left atrium appendage has to be excluded before ablation. In a study by Dorenkamp et al. the diagnostic accuracy of thrombus detection by MSCT was assessed as compared to transesophageal echocardiography in 329 patients scheduled for pulmonary vein isolation. MSCT showed low sensitivity (29%) and a high specificity (98%) among these patients with a prevalence of thrombus of 2.1% on echocardiography [64]. Accordingly,

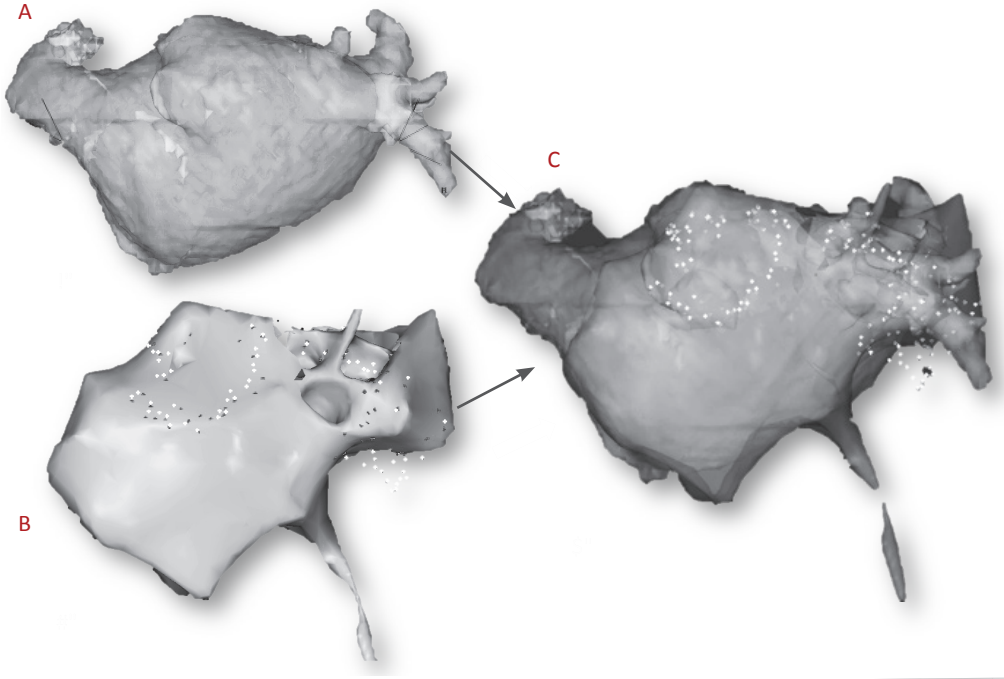

Figure 5. Posterior view of (A) multi-slice computed tomography reconstruction and (B) electro-anatomical mapping image of the left atrium. (C) Fusion of both images is helpful in anatomical orientation during ablation procedures. The white dotted points represent locations where electrical ablation was performed.

MSCT is an inappropriate modality for left atrial thrombus detection.

Three-dimensional MSCT is a reliable modality in imaging of the pulmonary veins before the catheter ablation of atrial fibrillation as it allows superior accuracy of pulmonary vein assessment as compared to echocardiography [65]. In the majority of cases four pulmonary veins are present. Nevertheless, anatomical variants have been described in pulmonary vein anatomy. A recent study showed a higher prevalence of a single pulmonary vein ostium on the left side in patients with atrial fibrillation as compared to a control group (33.7% versus 19.9% p=0.004) [66]. In addition, ostial diameters of the veins were larger in case of atrial fibrillation.

During the ablation procedure, fluoroscopy and electroanatomic mapping systems are used to identify sites for ablation. Integration of these modalities with MSCT has been introduced in the past years, and data demonstrating an improvement of the procedural outcomes become available. In a randomized study including 290 patients (145 patients with image integration and 145 with conventional mapping) the arrhythmia-free survival rate was significantly higher in image integration group as compared to conventional mapping group (88% versus 69%, p=0.017) (Figure 5) [67].

Finally, MSCT may help detect pulmonary vein stenosis after ablation. Indeed, pulmonary vein stenosis may occur in up to 10% of patients of catheter ablation of atrial fibrillation [62].

Evaluation in ventricular tachycardia ablation

Patients who suffer from appropriate implantable cardioverter defibrillator shocks for ventricular tachycardia despite treatment with antiarrhythmic medications may be candidates for radiofrequency ablation of ventricular tachycardia. Endocardial voltage mapping is normally applied for this purpose and has a limited ability to detect intramyocardial or epicardial scar. Moreover, suboptimal catheter contact can result in falsely low-voltage measurements. Accordingly, integration of MSCT images into the mapping systems may facilitate the ablation of ventricular tachycardia. A recent study demonstrated the feasibility of integration of MSCT derived data into mapping system in guiding catheter ablation [68]. It has also been demonstrated that the fusion of MSCT images with real time electroanatomic mapping data is accurate and may enhance the safety of epicardial catheter ablation procedures of ventricular tachycardia by establishing the relationship between the catheter tip and coronary arteries [69]. However, keeping in mind the emerging role of magnetic resonance imaging, nuclear imaging and intracardiac echocardiography for this purpose, more studies are necessary before MSCT may have a role in ablation of ventricular arrhythmias.

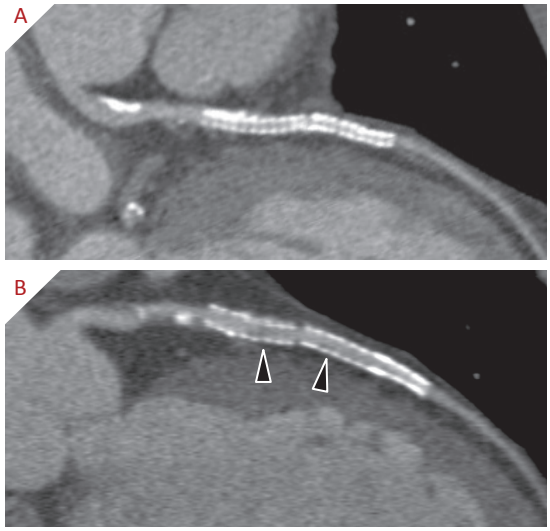

Figure 6. Coronary computed tomography angiography evaluation of coronary stents. Two patients, who were both evaluated for atypical complaints, already had a history of percutaneous coronary intervention. **(A)** Shows a left anterior descending coronary artery with two stents of a diameter of 2.75 mm. It is difficult to assess the presence of any stenosis within the stents; however, there is adequate runoff of contrast medium distal to the stents. **(B)** Three stents are visible in the LAD, two proximal stents of a diameter of 3.0 mm and a distal stent of a diameter of 2.75 mm. Some growth of neo-intima (arrowheads) can be assessed in both stents of 3.0 mm diameter.

Other applications of multi-slice computed tomography related to interventional cardiology

Evaluation of coronary artery stents

Detection of CAD in patients with a history of percutaneous coronary intervention might be clinically relevant. During the past several years the diagnostic accuracy of MSCT to detect in-stent restenosis remained a topic of investigation. Even with improved image quality with 64-slice MSCT and dual-source MSCT as compared to previous scanner generations, visualization of the lumen of coronary artery stents remains a challenge. Carrabba et al. performed a meta-analysis of nine studies with 64-slice MSCT including 598 patients and 978 stents [70]. In total, an average of 91% of stents was interpretable (the uninterpretable stents ranging from 0% to 42%). In the stents with good image quality, the sensitivity to detect in-stent restenosis as compared to ICA was 86%, whereas a NPV was 97%. Nevertheless, the PPV was limited to 70% indicating that the degree of in-stent restenosis is frequently overestimated with MSCT. The stent diameter has been reported to be a major predictor of stent evaluability (Figure 6) [71]. Accordingly, based on the available data, the experts currently consider MSCT appropriate for follow-up of asymptomatic patients after left main coronary artery stenting if a stent diameter is ≥3 mm [1].

Evaluation of coronary artery plaques

It is recognized that the morphology of coronary plaques plays an important role in the development of ACS [72]. Accordingly, the possibility to characterize coronary plaques with MSCT and investigation whether there is a correlation between certain

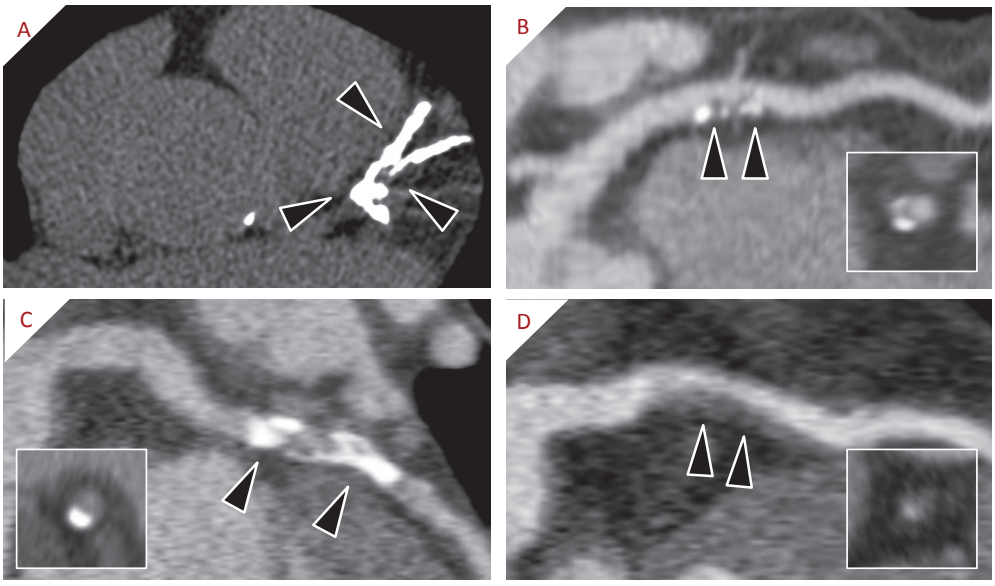

Figure 7. Visualization of coronary atherosclerotic plaques by computed tomography. (A) Visualization of coronary calcifications by means of contrast-free scan for coronary calcium scoring, demonstrating extensive calcification in the left anterior descending coronary artery and the first diagonal branch. **(B - D)** Curved multiplanar reconstructions of different types of coronary plaques, indicated by arrowheads, and with a cross sectional images. **(B)** A calcified plaque in the circumflex coronary artery, **(C)** a mixed plaque in the LAD, **(D)** a non-calcified coronary artery plaque in the LAD of a different patient.

findings and developing coronary events is clinically relevant.

Coronary atherosclerosis may be assessed with MSCT in two ways. Scans performed without contrast injection enable quantification of calcified component of coronary plaques (Figure 7). Calculation of Agatston score is the most widely accepted method for this purpose. Calcium scoring was performed in large patient populations and in different clinical settings ranging from asymptomatic patients to patients presenting with ACS. In general, coronary calcifications occur almost solely as a result of atherosclerosis and the amount of calcification roughly correlates to the extent of atherosclerotic burden [73]. Currently, data from large studies and with long follow-up are available on the value of calcium scoring in risk stratification of asymptomatic individuals. Indeed, a 6.8-year follow-up of 25,253 asymptomatic individuals by Budoff et al. demonstrated an increasing risk for all cause mortality with the increasing calcium scores [74]. Nevertheless, the correlation between individual lesion severity on ICA and the amount of coronary calcium is weak [73]. Moreover, absence of coronary calcium does not rule out coronary artery disease in symptomatic patients, particularly when young and presenting with acute symptoms [75].

Another way to visualize coronary plaques is MSCT coronary angiography, which

(due to intravenous injection of iodinated contrast) enables characterization of calcified and non-calcified plaque. Coronary plaques may be classified into non-calcified, calcified and mixed (containing both calcified and non-calcified components) (Figure 7). A recent meta-analysis including 22 studies demonstrated excellent diagnostic accuracy of MSCT for the detection of coronary plaques, with a sensitivity of 90% (95% CI 83%- 94%) and a specificity of 92% (95% CI 90%-93%) as compared to intravascular ultrasound [76]. However, if the plaques observed on MSCT angiograms are to be used for risk stratification and possibly going to be treated, a detection of plaque is not sufficient and a more detailed characterization of plaques is necessary.

The data become available on the prognostic value of coronary plaque characteristics with MSCT. In a study including 1,059 symptomatic patients who underwent diagnostic MSCT coronary angiography, the presence of coronary plaques with positive remodelling and plaques with low attenuation were associated with the development of ACS at 27 months of follow-up [77]. Based on these preliminary observations it may be concluded that coronary plaque imaging with MSCT may provide prognostic information. Nevertheless, further research is necessary to examine more detailed characterization of coronary plaque with MSCT before the information obtained on MSCT may be used for risk stratification or treatment purposes.

Future perspectives

Non-invasive evaluation of CAD with MSCT will remain a topic of investigation in the near future, especially with new scanning techniques allowing further reduction of radiation dose. With a low radiation exposure with the scans, the characteristics of potentially vulnerable coronary plaques will be explored, since the non-invasively obtained information on coronary atherosclerosis may potentially become a tool in patient risk stratification. Moreover, studies exploring prognostic value of MSCT with a long-term follow-up in various study populations are to be expected.

MSCT may provide additional information on anatomic relations of cardiac and adjacent structures in cardiac interventions, such as ablation in cardiac arrhythmias or procedures of cardiac valves. Prospective studies exploring the clinical advantages of use of MSCT need to further clarify the position of MSCT in the above procedures.

Importantly, appropriate use of MSCT which involves x-ray exposure will remain mandatory. Thereby optimal image quality with low radiation exposure will remain essential in cardiovascular applications of MSCT.

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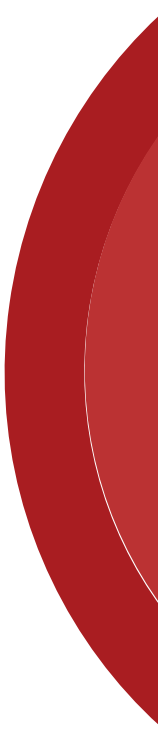

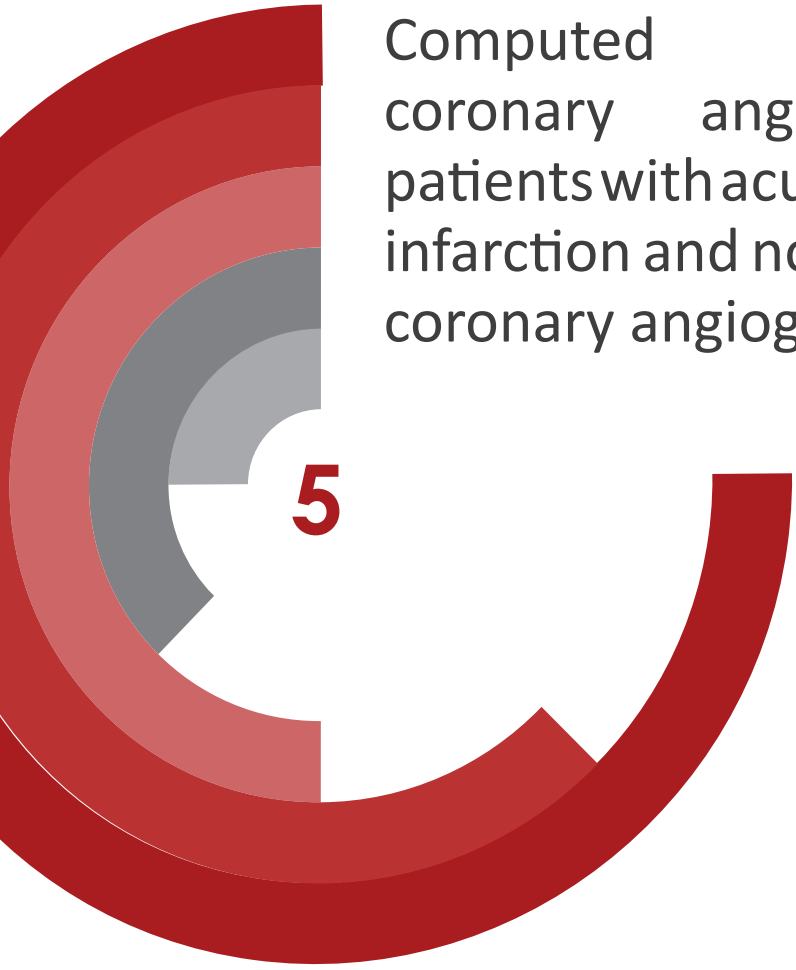

Computed tomography coronary angiography in patients with acute myocardial infarction and normal invasive coronary angiography

5

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Abstract

Aims: Three to five percent of patients with AMI, have normal coronary arteries on invasive coronary angiography (ICA). The aim of this study was to assess the presence and characteristics of atherosclerotic plaques on computed tomography coronary angiography (CTCA) in this group of patients.

Methods and Results: Thirty patients with AMI without visible coronary plaques on ICA underwent CTCA after ICA. Echocardiography was performed in the majority of patients. Twenty-eight patients presented with NSTEMI and two with STEMI. Mean age was 60.2 years and 23/30 were women. The prevalence of risk factors of CAD was low. 452 coronary segments were analysed. Eighty percent (24/30) had normal coronary arteries and twenty percent (6/30) had coronary atherosclerosis on CTCA. In case of atherosclerosis the median number of segments with plaque per patient was one. Echocardiography was normal in 12/26 patients, 11/26 patients had only wall motion abnormalities (WMA), 2/26 patients had WMA with minimal pericardial effusion and 1 patient had only minimal pericardial effusion.

Conclusion: Despite a diagnosis of AMI, 80% of patients with normal ICA showed no coronary plaques on CTCA. The remaining 20% had only minimal non-obstructive atherosclerosis. The previously proposed mechanism of AMI due to rupture of a non-obstructive-, invisible on ICA-, plaque could only account for a minority (20%) of this study population. Our data suggest that most patients either had AMI caused by a mechanism not involving plaque rupture or did not have an infarction at all.

Introduction

Acute myocardial infarction (AMI) usually results from thrombotic occlusion of a coronary artery due to a ruptured atherosclerotic plaque. However, some patients, about 3 to 5%[1,2] fulfil criteria for myocardial infarction but have angiographically normal coronary arteries (MINCA). The pathogenetic mechanisms that cause AMI in the patient with no visible coronary atherosclerosis on the invasive coronary angiography (ICA) are unknown. Alterations in the endothelium and/or of components in the blood promoting endothelial dysfunction and the formation of thrombotic occlusion have been suggested[3, 4]. According to Glagov[5] atherosclerotic plaques can cause outward remodelling of the coronary vessel without significant obstruction and therefore can be invisible on ICA. However, such plaques are prone to rupture and the development of an acute myocardial infarction[6]. If this is the case also in patients who show no visible changes on a conventional ICA, in spite of a diagnosis of myocardial infarction, remains unclear. Other proposed mechanisms are coronary dissection, embolism and vasospasm[7-9].

Although ICA has been the gold standard for the diagnosis of coronary artery disease, lumenography provides merely an image of the internal arterial lumen and lacks the capability to adequately depict the vessel wall with its developing atherosclerotic plaque. Previous studies analysing serial angiograms from patients presenting with ACS have suggested that in nearly two thirds of the culprit lesions, the coronary angiogram obtained a few months before the acute event demonstrated a non significant stenosis[10].

Imaging of the coronary vessels with computed tomography has been proposed as a method for qualitative imaging of vessel wall changes[11]. Computed tomography coronary angiography (CTCA) has a high negative predictive value, but tends to overestimate the degree of stenosis[12-14]. Previous studies have shown that CTCA is comparable to IVUS for classifying plaques[15-17]. The aim of the current study was to assess the presence and characteristics of atherosclerotic plaques on CTCA in patients with acute myocardial infarction who have a completely normal coronary angiogram on ICA. We hypothesise that plaques are present in patients with acute myocardial infarction and normal coronary arteries on ICA. This group of patients with acute myocardial infarction and angiographically normal coronary arteries is broadly recognized and described in several series. They are often described as being younger than the “classical” myocardial infarction patients and with lower burden of cardiovascular risk factors but the pathogenesis of this kind of presentation of myocardial infarction is still debatable.

Methods

Study design and population

This was a multi-center, prospective, descriptive study carried out in 3 hospitals in southeast Sweden, Linköping, Kalmar and Jönköping from November 2008 to January 2011. Patients were included at the local hospital where they presented with myocardial infarction and after they underwent an ICA. After the inclusion they were forwarded to University Hospital in Linköping for the CTCA part of the study. Inclusion criteria for the study were: myocardial infarction according to the ESC guidelines of 2007, with no visible atherosclerosis on ICA as assessed by two independent experienced operators[18]. The exclusion criteria were: inability to perform CTCA (contraindications to beta-blockers or nitroglycerine, allergy to contrast medium, pregnancy and permanent atrial fibrillation), renal dysfunction (creatinine clearance <60 ml/min) or risk factors for contrast induced acute kidney injury (treatment with metformin, high dose diuretics), recent major trauma, surgery or PCI, or the lack of informed consent.From November 2008 to January 2011, 30 patients were enrolled in the study. Clinical characteristics are displayed in Table 1. Twenty-eight patients presented with NSTEMI and two with STEMI. Mean age of the study population was 60.2 years (51.3 - 69.1) and 23/30 (77%) were female. 17/30 (57%) were previous or active smokers, 7/30 (23%) had hypertension, 5/30 (17%) had hypercholesterolemia and none had diabetes. There were 3/30 (10%) patients who were previously diagnosed with myocardial infarction. CTCA was performed within three days after ICA. Echocardiography was not part of the study protocol and was performed in routine clinical practice upon the discretion of the treating physician.

The study complies with the Declaration of Helsinki. Approval was obtained from the Regional Ethical Review Board in Linköping. All participants gave written informed consent.

Computed Tomography Coronary Angiography: image acquisition

CTCA was performed at the University Hospital Linköping using a 64-slice or a 128-slice dual source CT scanner (Somatom Definition or Somatom Definition Flash, Siemens Healthcare, Forchheim, Germany). During CTCA acquisition non-ionic contrast medium was administered, 60-50ml, 370 mg I/ml, 5ml/sec, Jopromid. Intravenous beta-blockers were administered if not contraindicated, for optimal image quality. Additionally, nitroglycerine was administered to all patients before the scan. Strategies to reduce radiation dose, including electrocardiogram gated tube current modulation, prospective triggering and reduction of tube voltage were used whenever feasible. The following scan parameters were used: 1. for 64 slice

Table 1. Patient characteristics

No. of patients	N=30	
Mean age , years	60.2 ± 8.9	
Males	7 (23%)	
Females	23 (77 %)	
Risk factors		
Previous or present smoker	17 (57 %)	
Median BMI	25 (23 –28)	
Diabetes mellitus	0 (0 %)	
Hypertension	7 (23 %)	
Hyperlipidaemia	5 (17 %)	
Previous myocardial infarction	3 (10 %)	
Previous stroke	0 (0 %)	
Clinical presentation		
NSTEMI	28 (93 %)	
STEMI	2 (7 %)	
Medication	on admission	at discharge
Acetyl salicyli c acid	4 (13 %)	28 (93 %)
Clopidogrel	1 (3 %)	22 (73 %)
Beta blocker	5 (17 %)	25 (83 %)
Calciumantagonist	1 (3 %)	2 (7 %)
ACEI/ARB	2 (7 %)	15 (50 %)
Statin	4 (13 %)	28 (93 %)
Diuretics	1 (3 %)	1 (3 %)
Laboratory results		
Creatinine on admission	75,0 (61,0 –81,3)	
Creatinine at 3 months follow -up	75,0 (64,0 –78,8)	
Peak Troponin I (ng/mL) (N=18)	1,6 (0,6 –6,3)	
Peak Troponin T (ng/mL) (N=5)	0,2 (0,1 –0,9)	
Peak hs -Troponin T (ng/L) (N=7)	873,0 (183,0 –1160,0)	
Number of patients with elevated Troponin	30 (100%)	
ApoA1	1,5 (1,3 -1,6)	
ApoB	0,9 (0,7 -1,1)	
Triglycerids	1,2 (0,8 -1,6)	
Cholesterol	5,2 (4,8 -6,1)	
HDL - cholesterol	1,6 (1,2 -2,1)	
LDL - cholesterol	3,1 (2,5 -3,9)	

The data are mean±SD, median, IQR, or numbers (%). ACEI = Angiotensin-converting enzyme inhibitor; Apo = Apolipoprotein; ARB = angiotensin receptor blocker; BMI = body mass index; IQR = interquartile range; HDL = high density lipoprotein; LDL = low density lipoprotein; NSTEMI = non ST-elevation myocardial infarction; SD = standard deviation; STEMI = ST-elevation myocardial infarction.

CT scanner: 64 x 2 slices with 0.6 mm collimation, gantry rotation time of 330 ms, tube voltage 100 or 120 mV, and effective tube current of 320 to 412 mAs; 2. for the 128-slice CT scanner: 128 x 2 slices with 0.6 mm collimation, gantry rotation time of 280 ms, tube voltage 100 or 120 mV, and effective tube current of 320 to 370 mAs.

Computed Tomography Coronary Angiography: image analysis

CTCA datasets were evaluated on a remote workstation with dedicated software (QAngio CT, Medis Medical Imaging Systems, Leiden, the Netherlands)[19]. Evaluation

Table 2. Findings on ICA, echocardiography and CTCA

Invasive coronary angiography	N=30
Normal coronary arteries	30 (100)
Wall irregulari ties	0 (0)
Coronary stenoses	0 (0)
Coronary anomaly	2 (7 %)
Aberrant Cx origin from right sinus Valsalva	1 (3.5%)
Aberrant RCA origin from left sinus Valsalva	1 (3.5%)
Echocardiography	N=26
Wall motion abnormalities	11 (42 %)
Wall m otion abnormalities and pericardial effusion	2 (8 %)
Pericardial effusion	1 (4 %)
Computed tomography angiography	N=30
Coronary arteries	
Normal coronary arteries	24 (80 %)
Coronary atherosclerosis	6 (20 %)
If atherosclerosis, number of segments with plaque	1 (1 –2)
Coronary anomaly (same as on ICA)	2 (7 %)
Other findings	
Pericardial thickening or effusion	9 (30 %)
Aortic valve calcification	2 (7 %)
Aortic calcifications	1 (3 %)
Aorta ascendens dila tation	1 (3 %)
Hiatal hernia	1 (3 %)
Liver cysts and post -infectious lung findings	1 (3 %)
Lung fibrosis	1 (3 %)
COPD	1 (3 %)
Nonspecific lung findings	2 (7 %)
Vertebral compression	1 (3 %)

The data are median, IQR, or numbers (%). IQR = interquartile range, ICA= invasive coronary angiography, CTCA: computed tomography coronary angiography

was performed side by side in consensus by two experienced observers blinded to baseline patient characteristics and ICA results. Lumen and plaque analysis were performed at a predefined window and level setting (window 900, level 250 Hounsfield units)[20]. If considered necessary, display settings were manipulated in order to achieve optimal discrimination of vessel lumen and plaque components and minimize blooming artifacts of calcified plaques. Coronary segments were visually scored for the presence of plaques. Seventeen segments were differentiated, according to a modified American Heart Association classification[21]. Tissue structures >1 mm^2 either within the coronary artery lumen or adjacent to the coronary artery lumen which could be discriminated from surrounding pericardial tissue, epicardial fat, or the vessel lumen, were defined as coronary plaques. Degree of stenosis of atherosclerotic lesions was quantified by visual estimation. Plaques with ≥50% luminal narrowing were classified as obstructive. Plaques were classified according to their composition into three types: 1. noncalcified plaque (plaques with lower density compared to contrast-enhanced lumen), 2. calcified plaque (plaques with high density structures compared to contrast-enhanced lumen), or 3. mixed plaque (noncalcified and calcified elements in single plaque). In addition, thickening of the pericardium and/or the presence of pericardial effusion was evaluated[22].

Troponin analysis

Three different troponin assay methods were used during the course of the study according to local routines. Troponin I (ULN: <0,04 µg/L), Troponin T (ULN: 0,01 µg/L) and hs-Troponin T (ULN: 15 ng/L).

Statistical analysis

Continuous variables are presented as mean±SD when normally distributed and as medians with interquartile range (IQR) when skewed. Categorical variables are presented as numbers and percentages. Statistical analyses were performed using SPSS version 20 (Chicago, IL).

Results

Clinical

Troponin levels of all patients were elevated (Table 1). Troponin I was used in 18 patients with mean peak value 1.6 µg/mL (IQR 0.6–6.3 µg/mL). Troponin T was used in 5 patients with mean peak value 0.2 µg/mL (IQR 0.1–0.9 µg/mL). hs-Troponin T was used in 7 patients with mean peak value 873.0 ng/L (183.0–1160.0 ng/L). ICA showed no atherosclerosis in all patients (Table 2). None of the patients had atrial

fibrillation/flutter or other type of supraventricular or ventricular tachycardia at presentation or during hospitalization.

Computed Tomography Coronary Angiography

The CTCA was performed a median of 3 days after ICA. A total number of 452 segments were analyzed. All coronary artery segments were of diagnostic image quality. A total of 24 patients had normal coronary arteries, and 6 patients had coronary atherosclerosis. In case of atherosclerosis, the median number of segments with plaque per patient was one. On CTCA thickening of pericardium or minimal pericardial effusion was present in 9 patients. Additional findings included coronary anomalies, aortic calcifications and dilatation, hiatus hernia and lung disorders (Table 2).

Echocardiography

Echocardiography was performed in 26 patients during hospitalization according to local routines. The echocardiographical examinations were reviewed once again during the analysis of study data but not based on a predefined protocol. A total of 12 patients had completely normal echocardiography results (Table 2). Eleven patients had only wall motion abnormalities, two patients had wall motion abnormalities with minimal pericardial effusion and one patient had only minimal pericardial effusion. Among patients with wall motion abnormalities there were three patients with wall motion abnormalities that could fit a takotsubo pattern (apical ballooning), one of these patients also had minimal pericardial effusion.

Pharmacological treatment

At the time of discharge from the hospital the majority of patients had been treated with acetyl salicylic acid, statin, beta-blocker and clopidogrel. Half of the patients were discharged with ACE-inhibitor or ARB. At the time of admission only a small percentage of patients had any medication (Table 1).

Discussion

In opposition to our hypothesis and previous study findings[23], we found that the majority of our patients (80%), who were diagnosed with AMI without visible atherosclerosis on a routine invasive coronary angiography, had totally normal coronary arteries on CTCA as well. The remaining 20% of patients had only minimal, one-segment, non-obstructive atherosclerosis. It appears unlikely that eccentrically remodelled atherosclerotic plaques that were invisible in ICA caused an AMI in

our patients since these plaques should be visible on CTCA[23]. The limited extent of atherosclerosis found in the remaining 20% of patients makes the plaque hypothesis less likely even in this subgroup.

In our population the majority of patients (27/30) were diagnosed with a first time myocardial infarction. The mean age was 60.2 years which is in accordance with previously observed age for first time AMI[24, 25]. The prevalence of risk factors was however lower from what was observed in large epidemiological studies[18] where the prevalence of diabetes, hypertension and hyperlipidaemia was 18.5%, 39.0% and 90% respectively. In our small study the prevalence was 0% for diabetes, 23% for hypertension and 17% for hyperlipidaemia. Most patients were women, 77% (23/30), which is in accordance with previous analyses where female sex was the strongest predictor of insignificant CAD in patients with NSTEMI[26]. The most frequent cause of AMI in similar female populations with non-obstructive CAD was previously found to be plaque rupture and ulceration[27]. However 80% of our population had no atherosclerosis at all (either on ICA or on CTCA), leaving mechanisms like vasospasm, dissection, embolism and impaired coagulation and fibrinolysis as possible causes of AMI. Even if these patients fulfilled diagnostic criteria for myocardial infarction, alternative explanations for chest pain and positive biomarkers as in myocarditis may have been overlooked. It has been shown before that up to 50% of patients with raised troponin, and unobstructed coronary arteries had myocarditis as diagnosed by cardiac MRI.[28]

Echocardiography was performed in the majority of patients (26/30). Fifty per cent of them (13/26) showed wall motion abnormalities (WMA), a finding that is compatible with a diagnosis of AMI but other conditions, like myocarditis, can have similar WMA. Among the patients with wall motion abnormalities there were 3 patients where apical hypokinesia was distributed over several coronary perfusion territories that could support the diagnosis of takotsubo cardiomyopathy. Minimal pericardial effusion was found in 3 patients, a finding that is not specific but it could be a sign of perimyocarditis.

Most patients were prescribed acetyl salicylic acid (93%) and a great proportion of them were sent home with dual antiplatelet therapy (73%), beta-blocker (83%) and statin (93%), thus being treated as “classical” AMI patients. We do know however, that patients with AMI and insignificant coronary atherosclerosis have a lower incidence of adverse outcomes compared with patients with significant coronary artery disease[26]. With their lower burden of risk factors in mind, such extensive medication may be inappropriate.

The important implications a diagnosis of acute myocardial infarction can have

for the psychological well-being of the patient[29, 30] for classification of risk in health insurance and the consequences for society because of sick leave, pension- and insurance claims[31], underscore the need for using additional imaging such as MRI in this group of patients.

In a recent study[32] where 152 patients with MINCA underwent cardiac MRI, the findings were normal in two thirds of the patients, 7% had signs of myocarditis and about 20% signs of myocardial necrosis. Amongst patients with normal MRI, 32% had typical clinical signs and symptom of Takotsubo cardiomyopathy and reversible wall motion abnormalities. Interestingly, the initial diagnosis of AMI was changed in two thirds of the patients after the MRI examination. These findings suggest that a significant part of this population actually has other diagnoses than AMI and more extensive evaluation than just coronary angiography is needed.

Study limitations

During the course of the study the three centers used different troponin assay methods excluding comparisons of the level of troponin rise. Another limitation of the study is that echocardiography was not part of the study protocol and it was left to the treating physician to decide if an echocardiographical examination was necessary and when to perform it. Though we finally had access to echocardiography in the majority of patients, the content of the examinations and the parameter analysis was not predefined.

Conclusions

Despite a diagnosis of AMI, based on ESC guideline criteria, the majority of patients with a completely normal ICA showed no coronary plaques on CTCA. A few patients had only minimal non-obstructive atherosclerosis. Our data suggest that most patients either had AMI caused by a mechanism not involving plaque rupture or did not have an infarction at all. Having in mind what consequences a diagnosis of AMI confers to the patient it is reasonable to extend the diagnostic evaluation of these patients to cardiac MRI for excluding myocarditis and/or intravascular imaging (optical coherence tomography or intravascular ultrasound) to provide information about some mechanisms of coronary damage (i.e. dissection).

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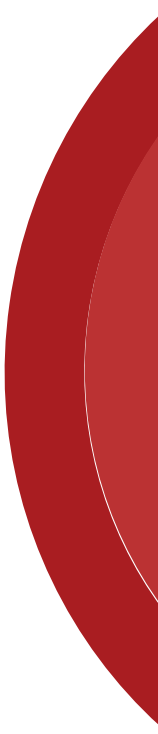

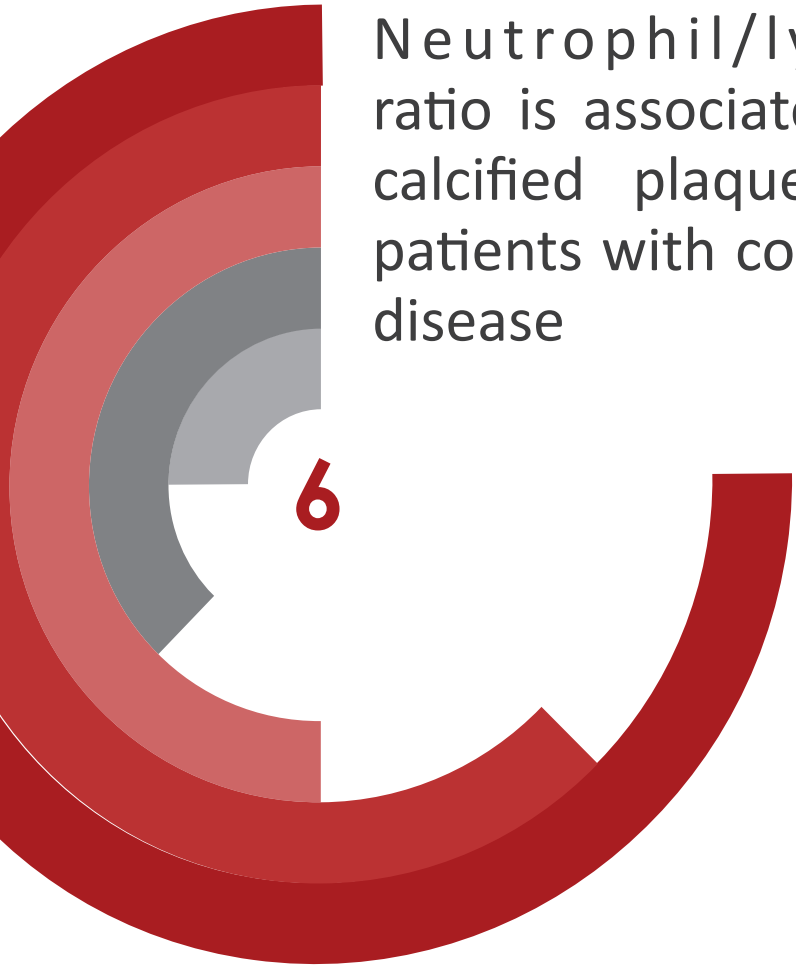

Neutrophil/lymphocyte ratio is associated with non-calcified plaque burden in patients with coronary artery disease

6

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Abstract

Background Elevations in soluble markers of inflammation as well as changes in leukocyte subset distribution are frequently reported in patients with coronary artery disease (CAD). Lately, the neutrophil/lymphocyte ratio has emerged as a potential marker of both CAD severity, as determined by invasive coronary angiography, and cardiovascular prognosis.

Objectives The aim of the study was to investigate whether neutrophil/lymphocyte ratio and other immune-inflammatory markers were related to plaque burden, as assessed by coronary computed tomography angiography (CCTA), in patients with CAD.

Methods Twenty patients with non-ST-elevation acute coronary syndrome (NSTE-ACS) and 30 patients with stable angina (SA) underwent CCTA at two occasions, immediately prior to coronary angiography and after three months. Atherosclerotic plaques were classified as calcified, mixed and non-calcified. Blood samples were drawn at both occasions. Leukocyte subsets were analyzed by white blood cell differential counts and flow cytometry. Levels of C-reactive protein (CRP) and interleukin(IL)-6 were measured in plasma. Blood analyses were also performed in 37 healthy controls.

Results Plaque variables did not change over 3 months, total plaque burden being similar in NSTE-ACS and SA. However, non-calcified/total plaque ratio was higher in NSTE-ACS, 0.25(0.09-0.44) vs 0.11(0.00-0.25), $p < 0.05$. At admission, levels of, monocytes, neutrophils, neutrophil/lymphocyte ratios, CD4+ T cells, CRP and IL-6 were significantly elevated, while levels of NK cells were reduced, in both patient groups as compared to controls. After 3 months, levels of, monocytes, neutrophils, neutrophil/lymphocyte ratios and CD4+ T cells remained elevated in patients. Neutrophil/lymphocyte ratios and neutrophil counts correlated significantly with numbers of non-calcified plaques and also with non-calcified/total plaque ratio ($r=0.403$, $p = 0.010$ and $r=0.382$, $p = 0.024$, respectively), but not with total plaque burden.

Conclusions Among immune-inflammatory markers in NSTE-ACS and SA patients, neutrophil counts and neutrophil/lymphocyte ratios were significantly correlated with non-calcified plaques. Data suggest that these easily measured biomarkers reflect the burden of vulnerable plaques in CAD.

Introduction

Coronary artery disease (CAD) is the leading cause of death in the western world. Although multifactorial in its origin, inflammatory and immunological events are considered to play central roles in initiation and progression of atherosclerotic plaques [1]. Indeed, elevations in soluble markers of inflammation as well as changes in leukocyte subset distribution are frequently reported in patients with CAD [2-5]. However, studies on relationships between markers of inflammation and severity of CAD have yielded disparate results [6-9].

In recent years, several studies have demonstrated the important role of neutrophils in all stages of atherosclerosis and plaque destabilization leading to acute coronary syndromes (ACS) [reviewed in 10]. Accordingly, neutrophil infiltration has been detected in very early stages of atherosclerosis as well as in shoulder regions of plaques prone to rupture [11,12]. Circulating neutrophil counts and neutrophil/lymphocyte ratios are emerging markers of the presence and severity of CAD [13-15]. Furthermore, they are independent predictors of mortality and cardiovascular events in high-risk groups and a broad range of CAD patients [14, 16-19].

CAD severity is not only a question about the extent of obstructive stenosis, but the risk of plaque rupture and ACS largely depends on plaque composition [20]. Coronary computed tomography angiography (CCTA) is a non-invasive method allowing accurate assessment of CAD [21]. In contrast to invasive coronary angiography (ICA), CCTA provides information about the vessel wall and composition of plaques in addition to degree of stenosis. CCTA may therefore provide valuable information about the burden of CAD with prognostic implications as well as assessing the morphological aspects of the disease process [22-24].

The identification of circulating immune-inflammatory markers that are associated with the atherosclerotic disease process in coronary arteries may provide additive information. The aim of the study was to investigate if neutrophil counts, neutrophil/lymphocyte ratio or other immune-inflammatory markers were related to plaque burden, as assessed by CCTA in patients with stable angina (SA) and ACS.

Materials and methods

Study design and population

This study is a single-center, prospective, pilot study. The study population consisted of 30 patients with SA, 20 patients with non-ST-elevation acute coronary syndrome (NSTE-ACS), and 37 healthy control subjects. In order to assess coronary atherosclerosis the patients underwent CCTA prior to ICA and revascularization (percutaneous coronary intervention (PCI) or coronary artery bypass grafting).

A follow-up CCTA was performed at three months following revascularization in order to reevaluate the plaque burden and plaque composition. Blood samples were collected at baseline (prior to CCTA and ICA), and at three months follow-up. Inclusion criteria were as follows: 1) patients with planned ICA due to SA, defined as clinically probable angina pectoris and positive exercise test or myocardial perfusion imaging; 2) NSTE-ACS, defined as unstable angina or non-ST-elevation myocardial infarction, according to universally accepted definitions [25, 26]. The exclusion criteria were: 1) inability to perform CCTA (contraindications to beta-blockers or nitroglycerine, allergy to contrast medium, pregnancy, permanent atrial fibrillation); 2) renal dysfunction (creatinine >150 µmol/L) or risk factors for contrast induced acute kidney injury (treatment with metformin, high dose diuretics); 3) known factors influencing immunological and inflammatory markers (active immunologic or inflammatory disease, infection with fever or use of antibiotics during the last 30 days, immunosuppressive treatment); and 4) major trauma, surgery or PCI in the last 30 days. The control subjects, randomly invited from the Swedish Population Register and representative for hospital recruitment area, were clinically healthy and received no medication. The study protocol was approved by the Regional Ethical Review Board in Linköping and written informed consent was obtained from all study participants. The study was conducted in accordance with the ethical guidelines set forth in the Declaration of Helsinki.

Coronary computed tomography angiography: image acquisition

Patients underwent CCTA within twenty-four hours after inclusion using a 16-slice multi-slice CT scanner or a 64-slice dual-source CT scanner (Sensation or Somatom Definition, Siemens Healthcare, Forchheim, Germany). During CTA acquisition non-ionic contrast medium was administered (Iomeron 400, Bracco, Altana, Pharma, Konstanz, Germany). Beta-blocker treatment (orally or intravenously) and nitroglycerine was administered to achieve optimal image quality. In order to reduce radiation exposure, electrocardiogram-gated current modulation was used in all patients. The following scan parameters were used: 1. For the 16-slice multi-slice scanner: 16 x 0.75 mm collimation, gantry rotation time of 375 ms, temporal resolution of 188 ms, tube voltage 100 or 120 kV, and maximal tube current of 650 mAs; 2. For the 64-slice dual-source CT scanner: 64 x 2 x 0.6 mm collimation, gantry rotation time of 330 ms, temporal resolution of 83 ms, tube voltage 100 or 120 kV, and maximal tube current of 560 mAs. Upon completion of the scan, images were reconstructed, if possible in several phases of the R-R interval, to obtain motion-free images of the coronary arteries.

Coronary computed tomography angiography: image analysis

Evaluation of CCTA images was performed on a remote workstation with dedicated software (QAngio CT, Medis Medical Imaging Systems, Leiden, the Netherlands) [27], side by side in consensus by two experienced observers blinded to baseline patient characteristics and ICA results. A predefined window and level setting (window 900 HU, level 250 HU) was used for analysis of lumen and plaque [27]. Coronary segments were differentiated into seventeen segments, according to a modified American Heart Association classification [28]. Segments of insufficient quality for evaluation were scored as non-evaluable and excluded from analysis. The CT scan was considered unevaluable if 2 vessels had 3 or more segments that were non-evaluable. Presence of plaques was visually assessed. Coronary atherosclerosis was defined as tissue structures >1 mm2 within or adjacent to the coronary artery lumen but distinctive from surrounding pericardial or epicardial tissue. Per segment, one coronary plaque was selected. The degree of luminal narrowing of the coronary artery was quantified visually, based on comparison of the luminal diameter of the plaque containing segment to the luminal diameter of the most normal-appearing site immediately proximal to the plaque. Plaques with ≥50% luminal narrowing were classified as obstructive. In addition, plaque composition was assessed. Three types of plaques were classified: 1) Non-calcified plaque (plaques with lower density compared to contrast-enhanced lumen), 2) calcified plaque (plaques with high density structures compared to contrast-enhanced lumen), or 3) mixed plaque (non-calcified and calcified constituents in single plaque) [30]. The number of any plaques (plaque burden), as well as plaques with different features was calculated per patient.

Invasive coronary angiography: image acquisition and analysis

Since the assessment of degree of stenosis with 16-slice CCTA is only moderately accurate, ICA was used to assess the obstructive plaque burden. ICA of left and right coronary arteries was performed in multiple views by using the transfemoral approach. Coronary segments were scored in the same manner as on CCTA images, and a diameter stenosis of ≥50% was classified as obstructive. Digital angiograms were analyzed off-line with dedicated software (Coronary Artery Analysis System 9, Pie Medical Imaging, Maastricht, The Netherlands). All segments >1.5 mm in diameter with a <100% diameter stenosis were measured on the angiograms. The contrast-filled non-tapered catheter tip was used for calibration. The proximal and distal reference vessel diameters and minimal lumen diameter of the suspected lesion were recorded. The percentage of diameter stenosis was calculated.

Blood sampling, biochemical analysis and flow cytometry

Venous blood samples were collected in vacutainer tubes (using sodium heparin as anticoagulant). Baseline blood samples of NSTE-ACS patients were received within 24 hours of hospital admission. For all CAD patients, the samples at baseline and at three months were collected prior to CCTA and ICA. Samples were centrifuged within 30 minutes to separate plasma, which then was stored immediately at -70◦C until analyzed. White blood cell differential counts were determined in whole blood by Cell-Dyn Sapphire™ (Abbot Diagnostics). Leukocyte subset distributions were analyzed in whole blood by flow cytometry as previously described [31,32]. Briefly, monoclonal antibodies against CD3, CD4, CD8, CD19, CD16 and CD56 were purchased from BD Biosciences, San José, CA, US. The antibodies were marked with one of 3 fluorochromes: fluorescein isothiocyanate, phycoerythrin and peridinin chlorophyll protein. The cells were identified by combinations as follows: CD3/CD4/CD8 (T helper cells and cytotoxic T cells), CD19 (B cells) and CD3/CD16/CD56 (NK cells). Whole blood and antibodies were incubated for 15 minutes at room temperature, thereafter erythrocytes were lysed with FACSä Lysing Solution (BD Biosciences) for 15 minutes at room temperature. Samples were analyzed on a FACSCanto II (BD Biosciences) equipped with 3 lasers, a blue 488 nm, a red 633 nm and a violet 405 nm. Analysis of samples was stopped when 10,000 cells were collected in the lymphocyte gate. Data were analyzed and subpopulations gated with FACSDivaä 6.1.2 software (BD Biosciences).

C-reactive protein (CRP) was measured in serum using a highly sensitive latex-enhanced turbidimetric immunoassay (Roche Diagnostics GmbH, Vienna, Austria) with a lower limit of detection of 0.03 mg/L. IL-6 levels in plasma were measured using an ELISA (R&D Systems Europe, Abingdon, United Kingdom) with a lower limit of detection of 0.48 pg/mL.

Statistical analysis

Categorical variables are presented as numbers (percentages) and were compared between groups using chi-square or Fisher's exact tests. When normally distributed, continuous variables are expressed as mean ± SD and were compared using one-way ANOVA and Students t-test for independent samples. When non-Gaussian distributed, continuous variables are presented as medians with 25^{th} and 75^{th} percentiles and were compared using the nonparametric Kruskal-Wallis test and Mann-Whitney-U test. Wilcoxon signed-rank test was used for paired comparisons. Bivariate correlations were performed to assess the associations between continuous variables. A p-value of <0.05 was considered statistically significant. Statistical analyses were performed

Table 1. Baseline clinical and biochemical characteristics of patients and controls

	SA N = 30	NSTE-ACS N = 20	Controls N = 37	P-value
Age (years)	64 ± 9	67 ± 10	64 ± 8	NS
Female	4 (13)	5 (25)	9 (24)	NS
Waist circumference (cm)	102 ± 11	99 ± 13	95 ± 9	**0.025**
Current or previous smoker	22 (73)	15 (75)	1 (3)	**<0.001**
Hypertension	21 (70)	7 (35)	0	**<0.001**
Diabetes mellitus	4 (13)	1 (5)	0	NS
History of MI	6 (20)	2 (10)	0	**0.019**
Statin treatment	24 (80)	6 (30)	0	**<0.001**
Plasma cholesterol (mmol/L)	5.1 ± 1.0	5.4 ± 1.3	5.5 ± 1.1	NS
Plasma LDL-cholesterol (mmol/L)	2.9 ± 0.8	3.2 ± 1.2	3.4 ± 0.9	NS
Plasma HDL-cholesterol (mmol/L)	1.2 ± 0.2	1.3 ± 0.4	1.4 ± 0.4	**0.042**
Triglycerides (mmol/L)	1.4 (1.1,2.1)	1.4 (1.2,1.7)	1.1 (0.9,1.6)	**0.037**

The data are presented as mean ± SD, median (25^{th}, 75^{th} percentile), or numbers (%).SA = stable angina; ACS = acute coronary syndrome; MI = myocardial infarction; LDL = low density lipoprotein, HDL = high density lipoprotein. NS = non-significant ($p > 0.05$).

using SPSS version 20 (Chicago, IL, USA) and STATA version 11.0 (College Station, TX, USA).

Results

Study population

During the study period from March 2006 till June 2010 a total of 64 patients were initially included in the study. Fourteen patients were excluded from analysis: 11 patients did not complete any CCTA examination and in 3 patients the CCTA was of non-diagnostic quality. Baseline clinical characteristics of all patients are listed in Table 1. Revascularization was performed in 22 of 30 SA patients (8 PCI and 14 CABG) and in 13 of 20 NSTE-ACS patients (9 PCI and 4 CABG) within the first two months after study inclusion. All CAD patients were treated with statin therapy after admission.

Coronary computed tomography angiography

Data of the baseline CCTAs are summarized in Table 2. A 16-slice CCTA was used in 10 and a 64-slice CCTA in 40 of the 50 patients. The total plaque burden (i.e. the total

number of any plaque) did not differ between SA and NSTE-ACS patients neither did plaque characteristics differ significantly between the patient groups. However, there was a trend towards more calcified plaques in SA patients, whereas NSTE-ACS patients tended to have more non-calcified plaques. As a measure of vulnerable plaques, the ratio between non-calcified plaques and total plaques was calculated. This ratio was significantly higher in ACS patients as compared to SA patients.

Follow-up CCTA at 3 months was available in 41 patients (26 SA and 15 NSTE-ACS patients), 9 performed by a 16-slice CCTA and 32 by a 64-slice CCTA. There were no differences in total plaque burden or plaque characteristics between baseline and 3 months. Total plaque burden and non-calcified plaque/total plaque ratio were 9 (5,11) and 0.16 (0.00, 0.26), respectively, in SA patients, and 8 (3,10) and 0.25 (0.09, 0.44), respectively, in NSTE-ACS patients, at 3 months.

Invasive coronary angiography

The findings on ICA are presented in Table 2. There was no difference in the number of coronary artery segments with obstructive lesions in SA patients as compared to NSTE-ACS patients. Strong positive correlations were found between the number of obstructive stenosis on ICA and total plaque burden as well as number of non-calcified plaques on CCTA ($r=0.694$, $p<0.001$, and $r=0.398$, $p=0.004$, respectively). On the other hand, the non-calcified plaque/ total plaque ratio was not associated with the number of obstructive lesions on ICA (r=0163, NS).

Immune-inflammatory markers

At admission, levels of leukocytes, neutrophils, monocytes, neutrophil/lymphocyte ratios, CD4+ T cells, and plasma levels of CRP and IL-6 were significantly elevated, while levels of NK cells were reduced, in SA and NSTE-ACS patients as compared to controls (Table 3). Plasma CRP in SA and NSTE-ACS patients declined over time reaching similar levels as control subjects at 3 months (0.9 (0.3,2.8), 0.7 (0.3, 1.9) and 0.7 (0.4,1.2) ng/mL, respectively, NS). Similarly, plasma IL-6 in SA and NSTE-ACS patients did not differ significantly from controls at follow-up (2.4 (1.1,2.9), 1.7 (1.2,3.1) and 1.4 (1.0,2.2) ng/mL, respectively, NS).

At 3 months, neutrophil counts were still significantly higher in SA and NSTE-ACS patients as compared to control subjects (4.0 ± 1.6, 4.0 ± 1.4 and 3.0 ± 1.0 $x10^9/L$, respectively, $p=0.006$), as were the neutrophil/lymphocyte ratios (2.0 ± 0.8, 2.2 ± 0.7 and 1.5 ± 0.7, respectively, $p=0.030$). Also, levels of monocytes and CD4+ T cells remained unchanged at 3 months whereas NK cell levels increased (data not shown). Of note, SA and NSTE-ACS patients did not differ significantly in any immune-inflammatory markers at 3 months.

Correlations of coronary computed tomography angiography and invasive coronary angiography with immune-inflammatory markers

Neutrophil/lymphocyte ratios and neutrophil counts correlated with numbers of non-calcified plaques (r=0.302, p=0.028 and r=0.327, p=0.017) and also with non-calcified plaque /total plaque ratio (r=0.403, p = 0.010 and r=0.382, p = 0.024, respectively), but not with total plaque burden on CCTA. Furthermore, neutrophil/ lymphocyte ratios and neutrophil counts did not correlate with number of obstructive lesions on ICA. Other leukocyte subsets did not show any correlations with any of the plaque variables, neither did CRP or IL-6 (data not shown).

Discussion

This study investigated associations between immune-inflammatory markers and plaque burden, as assessed by CCTA in patients with stable and unstable conditions of CAD. The main finding of this study was the consistent and significant correlation of neutrophil counts and neutrophil/lymphocyte ratios with numbers of non-calcified plaques and non-calcified plaque/total plaque ratio, but not with total plaque burden.

Neutrophils and neutrophil/lymphocyte ratios were significantly higher in both SA and NSTE-ACS patients as compared to controls, not only at admission but also at 3 months. The contribution of immune cells such as monocytes/macrophages and T cells to atherosclerosis and plaque progression has been firmly established

Table 2. Baseline coronary computed tomography angiography and invasive coronary angiography

	SA N = 30	NSTE-ACS N = 20	P-value
CCTA characteristics at admission Number of segments	15 (15,16)	16 (15,16)	NS
Total plaque burden	9 (6,11)	8 (6,10)	NS
Non-calcified plaque	1 (0,2)	2 (1,4)	NS
Mixed plaque	3 (1,5)	4 (1,6)	NS
Calcified plaque	3 (2,5)	2 (0,3)	NS
Non-calcified & mixed plaque	4 (3,7)	6 (2,8)	NS
Ratio non-calcified plaque/plaque burden	0.13 (0.00,0.25)	0.25 (0.10,0.44)	**0.049**
ICA characteristics at admission			
Number of segments	15 (15,16)	16 (15,16)	NS
Segments with significant stenosis (>50%)	3 (2,6)	2 (1,4)	NS
Segments without significant stenosis	12 (9,14)	14 (11,15)	NS

The data are presented as median (25^{th}, 75^{th} percentile). CCTA = coronary computed tomography angiography; ICA = invasive coronary angiography; SA = stable angina; ACS = acute coronary syndrome. NS = non-significant (p > 0.05).

Table 3. Baseline leukocyte subsets and plasma cytokines of patients and controls

	SA N = 30	NSTE-ACS N = 20	Controls N = 37	P-value
Leukocytes (x10^9/L)	7.1 ± 2.2	7.7 ± 2.3	5.5 ± 1.4	**<0.001**
Neutrophils (x10^9/L)	4.1 ± 1.5	4.5 ± 1.7	3.0 ± 1.0	**<0.001**
Monocytes (x10^9/L)	0.6 ± 0.2	0.6 ± 0.3	0.4 ± 0.1	**<0.001**
Lymphocytes (x10^9/L)	2.2 ± 1.0	2.4 ± 0.9	2.0 ± 0.6	NS
*CD19+ cells, % of lymphocytes	10 (7,12)	10 (8,15)	10 (7,14)	NS
*CD4+ cells, % of lymphocytes	49 (42,54)	50 (44,54)	39 (33,48)	**<0.001**
*CD8+ cells, % of lymphocytes	25 (20,31)	25 (17,31)	23 (19,29)	NS
*NK cells, % of lymphocytes	12 (9,18)	12 (7,15)	18 (11,28)	**0.004**
Neutrophil/ lymphocyte ratio	2.1 (1.4,2.4)	2.0 (1.5,2.5)	1.5 (1.2,1.9)	**0.024**
Plasma IL-6,	3.1 (2.0,5.7)	4.9 (2.9,8.0)	1.4 (1.0,2.2)	**<0.001**
Plasma CRP	1.2 (0.3,3.2)	3.2 (2.2,5.0)	0.7 (0.4,1.2)	**0.002**

The data are presented as mean ± SD or median (25^{th}, 75^{th} percentile). SA = stable angina; NSTE-ACS = non-ST-elevation acute coronary syndrome; CD19+ cells, B cells; CD4+ cells, T helper cells; CD8+ cell, cytotoxic T cell; NK = Natural killer; IL = interleukin; CRP = C-reactive protein. NS = non-significant ($p > 0.05$).

over the years [33]. However, while being the most abundant white blood cell in the circulation, neutrophils are rarely detected in atherosclerotic plaques and therefore have attracted less attention. Nevertheless, over the past couple of years numerous studies have lent support to an important role of neutrophils in all stages of atherosclerosis [10]. Neutrophil count and neutrophil/ lymphocyte ratio are emerging markers of presence and severity of CAD [13-15,34]. They are both independent predictors of mortality and future cardiovascular events in healthy populations, high-risk groups and a broad range of CAD patients [14,16-19, 35,36].

Neutrophil counts and neutrophil/lymphocyte ratios have been associated with the presence, severity and progression of coronary atherosclerosis as assessed by various modes of coronary imaging [14,15,37]. In a large cohort study of 3005 consecutive patients undergoing ICA for various indications, neutrophil count and neutrophil/ lymphocyte ratio correlated significantly with the number of diseased vessels [14]. Moreover, a higher neutrophil/lymphocyte ratio was associated with higher coronary calcium scores measured by multidetector CT in 849 clinically healthy individuals participating in a health promotion program [37]. However, neither ICA nor coronary calcium score, provide any information about the plaque composition. Correlations of neutrophil counts and neutrophil/lymphocyte ratios with plaque composition on CCTA have not previously been performed.

By using CCTA it has been possible to show that plaque morphology is an independent predictor of prognosis [22,24,38]. Several studies have found that non-calcified plaques are associated with worse outcomes [23,39,40]. Among 3,499 consecutive symptomatic SA patients who underwent CCTA, 1,102 subjects with non-obstructive CAD were prospectively followed for a mean of 78 months. The death rate of these patients was 3.1%, increasing incrementally from calcified plaque (1.4%) to mixed plaque (3.3%) to non-calcified plaque (9.6%) [23]. Our present data indicate that the absolute neutrophil count as well as the neutrophil/lymphocyte ratio, obtained by a white blood cell differential test, reflect the burden of high-risk plaques, rather than the crude number of plaques. Combining these easily available biomarkers and CCTA may be of supplemental value in the identification of patients with vulnerable atherosclerotic plaques. It opens up the potential for early selective treatment and prevention of future myocardial damage.

The increased levels of CD4+ T cells and dynamic changes of NK cells in the patient population of the present study have been described recently [32,41]. However, we did not find any association between these cell subsets and CCTA plaque characteristics. Neither were there any relationships between the well-established inflammatory markers, CRP and IL-6, and the CCTA findings. Only one previous CCTA study has investigated immune cells in relation to plaque burden and plaque composition. Kashiwagi et al found that an increased level of the proinflammatory CK14+ CD16+ monocyte subset, but not the total number of monocytes or CRP, is related to the presence of vulnerable plaques in patients with stable angina pectoris [42]. Unfortunately, we did not measure monocytes subsets, but the lack of association between the total number of monocytes, CRP and the presence of vulnerable plaques on CCTA was also found in our study.

A few studies have been performed in search of associations between other inflammatory markers and CCTA characteristics [43,44]. Bamberg et al determined the association between several plasma biomarkers and coronary plaque burden assessed by CCTA in 313 patients with acute chest pain who ultimately had no evidence of ACS [43]. Only 25% of study individuals were on statins and those with prior CAD were excluded. Interestingly, they found higher levels of CRP and oxidized LDL cholesterol and lower levels of adiponectin in patients with exclusively non-calcified plaques as compared to those with any calcified plaque or no plaque at all. In our study, 60% of all patients were on statin treatment at the time of inclusion. This may explain the absence of correlations between CRP and plaque characteristics, since statins are known to markedly reduce CRP levels [45]. Another study by Harada et al included 178 non-ACS acute chest pain patients, who underwent CCTA examination

[44]. In contrast to the study by Bamberg et al, they found an association between CRP and the presence of calcified rather than non-calcified plaques.

This study has some major limitations. First, the size of the study population was small. On the other hand, we included both SA and ACS patients, who were examined by CCTA and blood sample analysis prior to ICA and revascularization as well as in the stabilized phase at 3 months. Secondly, this study was performed between 2006 and 2009, first using a 16-slice and later a 64-slice CCTA. The 16-slice CCTA is less accurate than the 64-slice CCTA in measuring degree of stenosis and plaque composition. However, we did use ICA as well to assess obstructive and non-obstructive plaque burden and CCTA was performed twice in a majority of patients yielding identical results.

To conclude, both neutrophil counts and neutrophil/lymphocyte ratio were significantly correlated with non-calcified plaque burden and non-calcified plaque/total plaque ratio. The results highlight the potential utility of these easily measured cellular markers in the risk assessment and monitoring of CAD patients.

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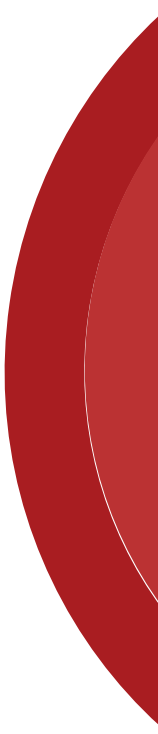

In Vivo Differentiation of Coronary Lesions with Noninvasive Computed Tomography Angiography and Invasive Intravascular Ultrasound as Compared to Optical Coherence Tomography

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Abstract

Purpose In vitro studies have shown the feasibility of coronary lesion grading with computed tomography angiography (CTA), intravascular ultrasound (IVUS) and optical coherence tomography (OCT) as compared to histology, whereas OCT had the highest discriminatory capacity. We investigated the ability of CTA and IVUS to differentiate between early and advanced coronary lesions in vivo, OCT serving as the standard of reference.

Methods Multimodality imaging was prospectively performed in 30 patients with NSTEMI. A total of 1083 cross-sections of 30 culprit lesions atherosclerotic plaque characteristics were assessed and co-registered among modalities. Fibrous and fibrocalcific plaque on OCT were defined as early plaque type, whereas lipid rich-plaque on OCT was defined as advanced plaque. To assess associations between each plaque type on CTA and IVUS with early or advanced plaque on OCT, odds ratios (OR) adjusted for clustering were calculated.

Results On cross-sectional level normal findings on CTA as well as on IVUS were associated with early plaque (OR: 0.09; $p<0.001$, and OR: 0.02; $p<0.001$, respectively). Non-calcified plaque on CTA was associated with advanced plaque (OR: 4.04, $p<0.001$) and a trend towards association between advanced plaque and calcified plaque on CTA was observed. The napkin ring sign on CTA was associated with advanced plaque (OR: 4.66, $p<0.001$). On IVUS fatty and calcified plaque were strongly associated with advanced plaque on OCT (OR: 151.93, $p<0.001$).

Conclusions In vivo coronary plaque characteristics on CTA and IVUS are associated with plaque characteristics on OCT. Of note, normal findings on CTA relate to early lesions on OCT. Nevertheless, multiple plaque features on CTA and IVUS are related to advanced plaques on OCT which may make it difficult to use qualitative plaque features to recognize advanced coronary plaques.

Introduction

Rupture of an atherosclerotic plaque with superimposed luminal coronary artery thrombosis is the most common cause of acute coronary events. Coronary artery plaques that are prone to rupture typically contain a large necrotic lipid-rich core and a thin overlying fibrous cap and are often referred to as vulnerable plaques [1-3]. Early detection of vulnerable plaques could potentially prevent coronary events. Computed tomography angiography (CTA) of the coronary arteries is a clinically established tool enabling non-invasive diagnosis of coronary artery disease (CAD) [4]. Besides stenosis assessment, it is possible to assess composition of atherosclerotic plaques with CTA and to a certain extent classify plaques. Although the presence of non-calcified plaque component on CTA was associated with the development of coronary events [5], the knowledge on the ability of CTA to discriminate between components of non-calcified plaque is still limited [6-8].

A multi-modality imaging strategy may enhance our understanding of coronary atherosclerotic lesions that are related to coronary events. Invasive coronary plaque characterization may be performed using intravascular ultrasound (IVUS) and optical coherence tomography (OCT)[9]. Whereas, IVUS is regarded as reference method for coronary plaque dimensions, OCT has shown a high diagnostic accuracy in characterization of plaques, however its penetration depth is limited [10, 11].

The feasibility of coronary lesion grading with CTA, IVUS and OCT as compared to histology was recently elegantly shown in an in vitro setting [12, 13]. OCT yielded the highest discriminatory capacity for advanced plaques as compared to histology [12]. Accordingly, in the study we investigated the ability of CTA and IVUS to differentiate between atherosclerotic plaque characteristics in vivo, with OCT serving as a standard of reference.

Methods

Thirty patients presenting with non-ST-elevation myocardial infarction (NSTEMI) were prospectively included in our study. Inclusion criteria for the study were patients with NSTEMI - chest pain suggestive for myocardial ischemia with typical electrocardiogram changes and a rise of (high sensitivity) troponin T - with a clinical indication for invasive coronary angiography (ICA) followed by reperfusion of the ischemia-related target lesion. Only native coronary artery lesions were included. Exclusion criteria were: persistent ST-segment elevation (>1mm in 2 or more leads), the need for emergency ICA with subsequent PCI or coronary artery bypass grafting (CABG), presence of cardiogenic shock, contraindication to CTA (estimated glomerular filtration rate <50 ml/min, known allergy to iodine contrast agents, cardiac rhythm

other than sinus rhythm, inability to lay supine or sustain a breath-hold for 15 seconds) and no informed consent. Dual-source CTA, IVUS and OCT were performed within 24 hours. The study was approved by the local ethics committee and was carried out according to the Declaration of Helsinki. Written informed consent was obtained from each participant.

Computed tomography angiography

Image acquisition

CTA was performed using a 64-slice dual-source computed tomography scanner (Somatom Definition, Siemens Healthcare, Frochheim, Germany). During CTA acquisition non-ionic contrast medium was administered (Iomeron 400, Bracco, Italy). Beta-blocker treatment (orally or intravenously) and nitroglycerine was administered to achieve optimal image quality. In order to reduce radiation exposure, electrocardiogram-gated current modulation was used in all patients. The following scan parameters were used for the 64-slice dual-source CT scanner: 64 x 2 x 0.6 mm collimation, gantry rotation time of 330 ms, temporal resolution of 83 ms, tube voltage 100 or 120 mV, and maximal tube current of 560 mAs. Upon completion of the scan, images were directly reconstructed in order to achieve motion-free images of the coronary arteries.

Image analysis

Evaluation of CTA images was performed on a remote workstation with dedicated software (QAngio CT, Medis Medical Imaging Systems, Leiden, the Netherlands) [14] in consensus by two experienced observers blinded to other imaging modalities. A predefined window and level setting (window 700 HU, level 200 HU) was used for analysis of lumen and plaque [7]. Display settings were manipulated for optimal analysis of vessel lumen and plaque characteristics, if deemed necessary. Analysis of presence and composition of atherosclerosis were performed on a per segment and on a per cross-section basis. In addition, in all other existing coronary segments the presence and composition of plaque as well as the degree of stenosis were also analyzed. Coronary segments were differentiated into seventeen segments, according to a modified American Heart Association classification [15].

Coronary atherosclerosis was defined as tissue structures >1 mm^2 within or adjacent to the coronary artery lumen but distinctive from surrounding pericardial or epicardial tissue. The degree of luminal narrowing of coronary artery lumen was quantified visually, based on comparison of the luminal diameter of the plaque-containing segment to the luminal diameter of the most normal-appearing site

immediately proximal to the plaque. Plaques with ≥50% luminal narrowing were classified as obstructive. The composition of plaque was classified to one of three types: 1. non-calcified plaque (plaques with lower density compared to contrast-enhanced lumen), 2. calcified plaque (plaques with high density structures compared to contrast-enhanced lumen), or 3. mixed plaque (non-calcified and calcified constituents in single plaque). In addition, the presence of thrombus was assessed on per segment level, which was defined as a homogenous non-calcified structure with irregular borders with a density of <55 HU [16].

On cross-sections with non-calcified plaque component (non-calcified and mixed plaques) the presence of napkin-ring sign (NRS) was scored as a plaque core with low CTA attenuation surrounded by a rim-like area of higher attenuation [17]. Non-calcified and mixed plaques were additionally classified according to their attenuation as low-attenuation (<30 HU) and higher attenuation (≥30HU) plaques [18].

Optical coherence tomography

Image acquisition

The C7 imaging system (St. Jude/LightLab Imaging Inc, MN, USA) was used to perform OCT acquisition. After crossing the culprit lesion with an angioplasty guide wire the OCT catheter (DragonFly catheter, St. Jude Medical/LightLab Imaging Inc, MN, USA) was advanced over the wire and placed distal to the lesion. In order to reduce vasospasm intracoronary nitro-glycerin was administered before OCT acquisition. OCT images were obtained during the intracoronary injection of 13 to 20 ml of contrast medium (Xenetix 300, Guerbet, France) at a rate of 3-4 ml/s and an automatic pullback at a rate of 20 mm/s.

Image analysis

Off-line IVUS and OCT cross-section analyses were performed using dedicated software (QIvus 2.1, Medis medical imaging systems, Leiden, the Netherlands) in consensus by two observers blinded to CTA, IVUS and OCT images of the same patient.

On OCT cross-sections morphological characterization of plaques was performed as follows (19): normal vessel wall; fibrous plaque, homogenous high-backscattering areas; lipid-rich plaque, signal poor regions with diffuse borders in >2 quadrants; fibrocalcific plaque, fibrous plaques with calcific areas (well-delineated, low back-scattering heterogenous regions). Thin cap fibro-atheroma (TCFA) was defined as a lipid-rich plaque with an overlying fibrous cap with a thickness of ≤65 µm (20). When a cross-section showed various plaque types, the most advanced plaque type

was assigned in the following order: lipid-rich plaque > fibrocalcific plaque > fibrous plaque. For comparisons we categorized plaques in early and advanced plaques. Based on associations found in the ex vivo study by Maurovich-Horvat et al. absence of plaque, fibrous and fibrocalcific plaques were defined as early plaques and lipid-rich plaques were defined as advanced plaques [12]. Plaque rupture was identified as the presence of fibrous-cap discontinuity with a cavity formation in the plaque [21]. The presence of thrombus was visually assessed. Intracoronary thrombus was defined as a mass protruding the coronary vessel lumen discontinuous from the vessel wall.

Intravascular ultrasound

Image acquisition

After OCT acquisition, the IVUS catheter (40MHz Atlantis SR pro catheter, Boston Scientific, MA, USA) was advanced to perform IVUS acquisition. The iLab ultrasound imaging system (Boston Scientific, MA, USA) was used to perform IVUS acquisition.

Image analysis

Assessment of IVUS cross-sections was performed in accordance with the working group on intracoronary imaging of the European Society of Cardiology using the following criteria [22]: normal vessel wall, <0.3 mm of intima-media thickness; vessel wall containing plaque, ≥0.3 mm of intima-media thickness; fibrous plaque, >2 quadrants constituted by tissue, with echoreflectivity higher than that of the adventitia; fatty plaque, >2 quadrants constituted by tissue, with echoreflectivity lower than that of the adventitia; fibrocalcific plaque, <2 quadrants of total calcified arc; and calcified plaque, >2 quadrants of total calcific arc.

Image co-registration

Anatomic landmarks, such as side-branches and/or calcifications were used to align CTA, IVUS and OCT images and co-registered cross-sections. Multiplanar CTA reconstructions were created perpendicular to the vessel centerline. At first a visual co-registration was performed to align CTA, IVUS and OCT longitudinal segments. Subsequently, dedicated software (Matcher, Medis Medical Imaging Systems, Leiden, the Netherlands) was used to confirm the performed visual co-registration. Where feasible, the co-registration was revised in order to achieve the best-matched result of the three imaging modalities. Anatomic landmarks were used to match the rotational direction of the cross-sections of CTA, IVUS and OCT.

Statistical analysis

Normally distributed continuous variables are presented as mean±SD. Continuous variables with skewed distribution are presented as medians with 25th and 75th percentile. Categorical variables are presented as numbers and percentages. Group differences were tested using ANOVA test, Pearson χ2, or Fishers exact test where appropriate.

Crude associations of each category of CTA and IVUS with early or advanced plaque defined by OCT were assessed by calculating odds ratios (OR) for each category with the odds of normal plaque serving as reference. An OR >1.0 indicated an increased probability of a lesion being advanced, and an OR <1.0 indicated an increased probability of a lesion being early. Significance of these crude associations was tested using the Fishers exact test. Additionally the associations were recalculated accounting for clustering effect within lesions by using multilevel mixed effect modeling (STATA procedure *xtmelogit*). Bonferroni correction was used because of multiple testing. For each modality the plaque categories were recoded as a new, single variable. For CTA the new variable had three degrees of freedom and for IVUS the new variable had four degrees of freedom. A mixed effects logistic regression for each modality was fitted with the category normal plaque serving as the reference category.

Statistical significance was defined as p-value of <0.05. Statistical analyses were performed using STATA version 11 (College Station, TX, USA).

Results

Study population

A total of thirty patients with NSTEMI were included in the study and multi-modality imaging was performed in 30 culprit lesions.

A total of 1083 cross-sections from 30 culprit segments were available for analysis. The mean number of available cross-sections was 36 per patient. Baseline characteristics of the population are presented in Table 1. The mean age was 68.3 years old, and half were of male gender.

Computed tomography angiography

On lesion level, the majority of the culprit coronary plaques (17; 56.7%) were classified as mixed plaques, whereas 5 (16.6%) were classified as non-calcified plaques and 8 (26.7%) were classified as calcified plaques. Thrombus was scored in 9 (30.0%) of the culprit segments.

On frame level, of the 1083 coronary CTA cross-sections, 257 (23.7%) were classified

Table 1. Patient characteristics

	N = 30
Demographics	
Age (years)	68.3±10.7
Gender (male)	17 (56.7)
Body mass index (kg/m2)	26.8 [24.4-28.9]
Medical history	
Hypertension	16 (53.3)
Diabetes	10 (33.3)
Hypercholesterolaemia	10 (33.3)
Current or previous smoker	13 (43.3)
Family history	8 (26.7)
Previous MI	4 (13.3)
Previous PCI	6 (20.0)
Previous CABG	2 (6.7)
Previous CVA	5 (16.7)
Laboratory values	
CK maximum (mg/L)	164.0 [86.5 - 301.0]
CKMB maximum (mg/L)	21.0 [17.0 - 33.0]
hs-TroponinT maximum (ng/L) [n=9]	192 [51 - 368]
Troponin T maximum (µg/L) [n=21]	0.10 [0.03 - 0.20]
Cholesterol (mmol/L)	4.9 [4.1 - 5.6]
HDL - Cholesterol (mmol/L)	1.4 [1.1 - 1.6]
LDL - Cholesterol (mmol/L)	3.1 [2.2 - 3.4]
Computed Tomography Coronary Angiography characteristics	
Number of segments	15 [14 - 16]
Plaques	8.5 [6.0 - 11.0]
Non-obstructive plaques	6.0 [5.0 - 8.3]
Obstructive plaques	2.0 [1.0 - 3.0]
Non-calcified plaques	1.0 [0 - 1.3]
Mixed plaques	2.0 [0 - 4.0]
Calcified plaques	4.5 [1.0 - 7.0]

The data are mean±SD, median, IQR, or numbers (%). CABG = coronary artery bypass graft; IQR = interquartile range; RCA = right coronary artery; LAD = left anterior descending artery; LCx = left circumflex artery; SD = standard deviation.

as normal, 360 (33.2%) as showing non-calcified plaque, 178 (16.4%) as showing mixed plaque, and 288 (26.7%) as showing calcified plaque. A total of 28/538 (5.2%) cross-sections exhibited a NRS.

Optical coherence tomography

On lesion level, thrombus was detected in 20 (66.7%), plaque rupture in 25 (83.3%), and TCFA in 13 (43.3%) of the culprit lesions.

On frame level, of the 1083 OCT cross-sections, 35 (3.3%) were classified as normal, 296 (27.3%) as fibrous plaques, 467 (43.1%) as fibrocalcific plaques and 285 (26.3%) as lipid-rich plaques. Thrombus was present in 97 (9.0%) frames. TCFA was present in 24 (2.2 %) frames.

Intravascular ultrasound

On lesion level, characteristics visualized by IVUS where as follows: thrombus was detected in 8 (26.7%) culprit segments, and rupture was detected in 13 (43.3%) plaques.

On frame level, of the 1083 IVUS cross-sections, 45 (4.2%) were classified as normal, 380 (35.1%) as fibrous plaques, 369 (34.0%) as fibrocalcific plaques, 173 (16.0%) as calcified plaques, and 116 (10.7%) as fatty plaques. Thrombus was detected in 21 (1.9%) cross-sections.

Table 2. Association between thrombus on OCT and plaque characteristics on coronary CT angiography and IVUS

		Thrombus on OCT	No thrombus on OCT
Segment level		**(n=20)**	**(n=10)**
CT			
	Thrombus	9 (45.0)	0 (0)
	NRS	5 (25.0)	0 (0)
	HU <30	10 (50.0)	1 (10.0)
	Non-calcified	2 (10.0)	3 (30.0)
	Mixed	13 (65.0)	4 (40.0)
	Calcified	5 (25.0)	3 (30.0)
IVUS			
	Thrombus	6 (30.0)	2 (20.0)
Cross-sectional level		**(n=97)**	**(n=986)**
CT			
	Thrombus	20 (20.6)	7 (0.7)
	NRS	16 (16.5)	12 (1.2)
	HU <30	19 (19.6)	11 (1.1)
	Non-calcified	52 (53.6)	308 (31.2)
	Mixed	23 (23.7)	155 (15.7)
	Calcified	22 (22.7)	266 (26.9)
IVUS			
	Thrombus	13 (13.4)	8 (0.8)
	Fibrous	21 (21.7)	359 (36.4)
	Fibrocalcific	31 (31.96)	338 (34.3)
	Calcified	25 (25.8)	148 (15.0)
	Fatty	20 (20.6)	96 (9.7)

The data are numbers (%). CTA = computed tomography angiography; HU = Houndsfield Units; IVUS = intravascular ultrasound; NRS = napkin ring sign; OCT = optical coherence tomography; TCFA = thin-cap fibroatheroma.

Comparison between computed tomography angiography versus optical coherence tomography

On per lesion basis, culprit lesions were grouped based on the presence of thrombus on OCT (Table 2). On CTA, thrombus and NRS were only observed in the groups of lesions with thrombus on OCT, but only in a limited number of lesions. Moreover, limited correlation was observed between the low attenuation value on CTA and the presence of thrombus on OCT. No correlation was observed between the plaque type on CTA and the presence of thrombus. The sensitivity for CTA to detect thrombus was 45% (9/20) and the specificity was 100% (10/10).

On per frame basis, thrombus was visible in only 21% of CTA frames with thrombus on OCT (Table 3). The same limited association was observed between the presence of thrombus on OCT and the NRS and low attenuation plaque.

Normal findings on CTA were associated with early plaque on OCT (Table 4). On the contrary, non-calcified were associated with advanced plaque on OCT and calcified plaques showed trend toward association with advanced plaque. Moreover, NRS was significantly associated with advanced plaque on OCT (OR: 4.66 p<0.001).

Table 3. Plaque composition as assessed at coronary CT angiography and IVUS within atherosclerotic plaque categories as assessed at OCT

	OCT				
	Associated with early lesions				Associated with advanced lesions
Modality and plaque composition	**Normal**	**Fibrous**	**Fibrocalcific**	**Total (all 3)**	**Lipid-rich**
CT					
Normal	23 (9.0)	152 (59.1)	18 (7.0)	193 (75.1)	64 (24.9)
Non-calcified	10 (2.8)	108 (30.0)	106 (29.4)	224 (62.2)	136 (37.8)
Mixed	0 (0)	18 (10.1)	115 (64.6)	133 (74.7)	45 (25.3)
Calcified	2 (0.7)	18 (6.2)	158 (79.2)	248 (86.1)	40 (13.9)
Total	35	296	378	798	285
CT additional information					
Napkin ring sign	0 (0)	1 (3.6)	10 (35.7)	11 (39.3)	17 (60.7)
IVUS					
Normal	30 (66.7)	13 (28.9)	0 (0)	43 (95.6)	2 (4.4)
Fibrous	5 (1.3)	233 (61.3)	51 (13.4)	289 (76.0)	91 (24.0)
Fibrocalcific	0 (0)	44 (11.9)	256 (69.4)	300 (81.3)	69 (18.7)
Calcific	0 (0)	0 (0)	148 (85.5)	148 (85.5)	25 (14.5)
Fatty	0 (0)	6 (5.2)	12 (10.3)	18 (15.5)	98 (84.5)

The data are numbers (%). CTA = computed tomography angiography; HU = Houndsfield Units; IVUS = intravascular ultrasound; OCT = optical coherence tomography.

Table 4. Association of coronary CT angiography and IVUS with lesions on OCT which are associated with early and advanced lesions on histologic examination

Modality and plaque composition		OCT						
		Total	Associated with early lesions	Associated with advanced lesions	Crude analysis		Accounted for clustering	
					OR	p-value	OR	p-value **
CT								
	Normal	257 (23.7)	193 (75.1)	64 (24.9)	0.33*	0.571	0.09*	0.000
	Noncalcified	360 (33.2)	224 (62.2)	136 (37.8)	1.83	0.000	4.04	0.000
	Mixed	178 (16.4)	133 (74.7)	45 (25.3)	1.02	0.780	1.91	0.098
	Calcified	288 (26.6)	248 (86.1)	40 (13.9)	0.49	0.000	2.16	0.049
IVUS								
	Normal	76 (6.6)	43 (95.6)	2 (4.4)	0.05*	0.000	0.02*	0.000
	Fibrous	396 (34.2)	289 (76.0)	91 (24.0)	6.77	0.219	5.61	0.116
	Fibrocalcific	377 (32.6)	300 (81.3)	69 (18.7)	4.94	0.000	6.27	0.087
	Calcific	180 (15.6)	148 (85.5)	25 (14.5)	3.63	0.000	9.47	0.030
	Fatty	128 (11.0)	18 (15.5)	98 (84.5)	117.06	0.000	151.93	0.000

*Odds of the reference category is given
** Bonferroni correction performed
The data are numbers (%). CTA = computed tomography angiography; IVUS = intravascular ultrasound; OCT = optical coherence tomography; OR = odds ratio.

Comparison between grayscale intravascular ultrasound versus optical coherence tomography

On lesion level, a very limited correlation between the presence of thrombus on OCT and IVUS was observed (Table 2). For IVUS the sensitivity and specificity were 30% (6/20) and 80% (8/10), respectively.

On frame level, the correlation between the presence of thrombus on OCT and the findings on IVUS was even lower than on lesion level (Table 2).

Normal findings on IVUS were associated with early plaque on OCT. On the contrary, calcified plaque was associated with advanced plaque on OCT, whereas fatty plaque on IVUS was strongly associated with advanced plaque on OCT (Tables 3 and 4).

An example of findings on all three imaging modalities is presented in Figure 1.

Discussion

In this in vivo study in patients with NSTEMI describing a systematic assessment of coronary lesions using CTA and IVUS compared to OCT as a standard of reference, the findings may be summarized as follows: [1] Normal findings on CTA and IVUS are associated with early plaque as defined by OCT; [2] Non-calcified plaque, NRS and to a lower extent calcified plaque on CTA as well as fatty and calcified plaque on IVUS are significantly associated with advanced plaque as defined by OCT.

In accordance with ex vivo studies, the absence of plaque on CTA was correlated to

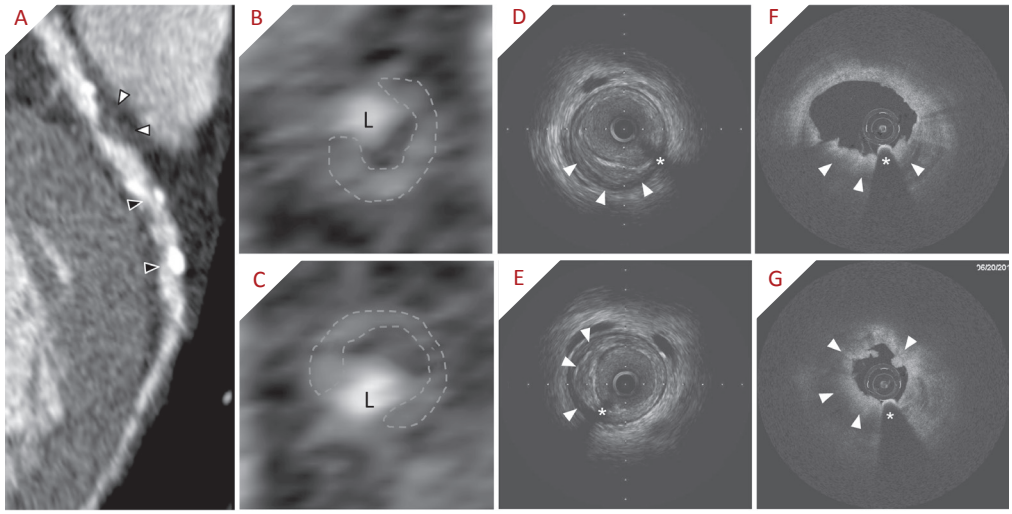

Figure 1. An example of a lesion in left anterior descending artery with corresponding cross-sections on computed tomography, intravascular ultrasound and optical coherence tomography Multiplanar reformatted image of the left anterior descending coronary artery (A) with a mixed plaqe with large non-calcified portion (white arrowheads) and some calcified plaques more distally (black arrowheads). The cross-sectional computed tomography images (B, C) show a coronary plaque with napkin-ring sign attenuation pattern (L = lumen). The circumferential outer rim (dashed blue line) has a higher contrast attenuation as compared to the inner part of the plaque. Intravascular ultrasound (D, E) images show a predominantly fibrous plaque (white arrowheads). Optical coherence tomography (F, G) of the corresponding cross-sections shows (mural) thrombus in both positions (white arrowheads).
* represents the shadow of the guidewire.

early plaque on OCT [12, 13]. Indeed, Maurovich-Horvath et al. have recently observed in an experimental model that normal findings by CTA were strongly associated with early atherosclerotic plaque on histological examination (OR: 0.02; p<0.001) [12]. The finding is line with prospective clinical studies which have shown that the absence of CAD on CTA resulted in no coronary events during follow-up [23-25]. This finding further supports the application of coronary CTA for exclusion of CAD. Moreover, correlation between certain coronary plaque characteristics on CTA and advanced lesions on OCT has been observed. A significant correlation between non-calcified plaque on CTA and advanced plaque on OCT has been found in our dataset, which is contrary to the findings in the previous ex vivo study where correlation between advanced lesions on histology and mixed plaque on CTA was observed [12]. From a clinical point of view both types of plaques (non-calcified and mixed) were related to coronary events on follow-up [5, 23, 26-28]. Moreover, the difference in the findings may be related to the different reference standard used in our study (OCT versus histopathology). Another interesting observation is the correlation between the NRS on CTA and advanced coronary plaque. Previous studies have shown the relation between the NRS and high-risk coronary plaques [13, 29-31]. In a study with donor hearts the specificity of NRS to identify histopathologically advanced lesions and TCFA was as high as 99% [13]. Moreover, another study demonstrated that the presence of NRS within a coronary

plaque is strongly associated with future cardiac events independent of other high-risk CTA features, such as positive remodeling and low attenuation plaque [32]. Nevertheless, we have also observed a trend toward correlation between advanced lesions on OCT and calcified plaque on CTA. The same finding has been reported in a study by Maurovich-Horvat et al.[13]. Since multiple coronary plaque features are related to advanced coronary lesions on OCT or histology as a standard of reference, it may be difficult to use qualitative coronary plaque features on CTA for further risk stratification.

Similar correlations between coronary plaque features and OCT were observed also on IVUS. Indeed, normal findings on IVUS were related to early lesions on OCT, whereas a strong correlation between the fatty plaque on IVUS and advanced plaque on OCT was observed. Also, similar to the findings on CTA calcified plaque on IVUS was related to advanced lesions. The findings are somewhat discrepant from the findings in a previous ex vivo study [12], which could be related to different classification scheme applied in our study and a small sample size.

In the present study we also assessed the ability of CTA and IVUS with inferior spatial resolution as compared to OCT to characterize non-calcified plaque component, including the assessment of thrombus. Thrombus was present in 66.7% of culprit lesions evaluated with OCT, which corresponds well to the prevalence of thrombus in culprit lesions of NSTEMI patients using OCT (65 – 68%) [33, 34]. Nevertheless, thrombus could only be identified in 30% of culprit lesions on IVUS. Our results correspond with the findings by Kubo et al., who reported the diagnostic accuracy for detection of thrombus by IVUS to be as low as 33% as compared to OCT and coronary angioscopy [21]. Regarding CTA, there are only a few reports available on the detection of coronary thrombus [16]. As expected, the accuracy of thrombus detection on CTA in our study was as low as 45%. Moreover, no other plaque features were associated with intracoronary thrombus on IVUS and CTA either on segmental or frame level (Table 2), which further emphasizes the limitation of IVUS and CTA to characterize non-calcified tissue. Accordingly, the presence of thrombus on IVUS and CTA should be interpreted with caution.

Our results should be interpreted in the context of several limitations. First, an important limitation of this study is the lack of histopathological data, however this study was designed as an in vivo study. Second, as the used modalities are different from the technical point of view, the plaque classification schemes were also different. Therefore direct comparison of plaque characteristics was difficult to perform. Finally, performing a complex multi-modality imaging study is difficult due to the costs and logistical difficulties. Therefore only a small number of patients could

be recruited to participate in the study resulting in a limited sample size.

In conclusion, in vivo coronary plaque characteristics on CTA and IVUS are associated with plaque characteristics on OCT. Of note, normal findings on CTA relate to early lesions on OCT. Nevertheless, multiple plaque features on CTA and IVUS are related to advanced plaques on OCT which may make it difficult to use qualitative plaque features to recognize advanced coronary plaques in clinical practice. Future studies in bigger samples are necessary to confirm the findings.

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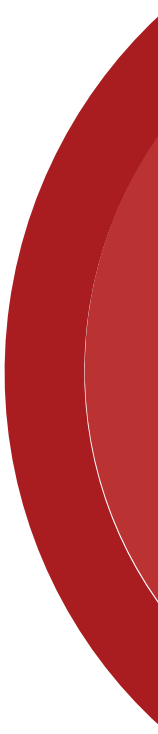

The Feasibility of Optical Coherence Tomography Guided Thrombus Aspiration in Patients With Non-ST-Elevation Myocardial Infarction After Initial Conservative Therapy – a Pilot Study

8

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During percutaneous coronary intervention (PCI) in patients with acute coronary syndrome balloon inflation or stent deployment leads to fragmentation of thrombus causing microembolization and myocardial damage [1]. Manual thrombus aspiration (TA) allows effective retrievement of thrombus in 70% to 80% of patients with ST-elevation myocardial infarction (STEMI) and non-ST-elevation myocardial infarction (NSTEMI) [2,3]. TA use in STEMI patients enhances myocardial perfusion, reduces the incidence of microvascular obstruction and improves prognosis [2,4,5]. However, data on TA in NSTEMI patients are scarce. One small study investigating TA in NSTEMI patients who underwent early revascularization demonstrated that TA is associated with high retrieval rates of thrombus with a marked reduction in Thrombolysis In Myocardial Infarction (TIMI) thrombus score and an increased rate of TIMI-flow grade 3 [3]. No data are available on thrombus aspiration in initially conservatively treated NSTEMI patients. TA is frequently performed when thrombus is visible on coronary angiography (CAG) although it is known that its accuracy in thrombus detection is limited. Intravascular imaging with optical coherence tomography (OCT) enables superior assessment of thrombus [6]. In this pilot study we investigated the efficacy of TA involving imaging with OCT in patients presenting with NSTEMI after initial conservative therapy.

Thirty NSTEMI patients who were conservatively treated the first 72 hours and had a clinical indication for PCI were prospectively included. Exclusion criteria were: electrocardiographic ST-segment elevation, hemodynamic instability, renal dysfunction (serum creatinine >2,26 mg/dL), and inability to perform OCT (TIMI flow grade 0 or 1, target vessel diameter <2.5 mm). Before PCI, all patients were treated with low molecular weight heparin, aspirin, and clopidogrel [7]. PCI was performed in the following order: first, OCT acquisition was performed (C7 imaging system, DragonFly catheter St. Jude/LightLab Imaging); second, TA was performed (6F Export Aspiration Catheter, Medtronic), followed by a second OCT acquisition; third, the culprit lesion was treated with stenting, followed by a third OCT acquisition. Effective TA was defined as the presence of atherothrombotic material in the aspirated samples and the material was analyzed as previously described [2].

Angiographic analysis involved assessment of TIMI flow grade and TIMI thrombus score. OCT images were analyzed off-line using dedicated software (QIvus 2.1, Medis medical imaging systems). Identical regions of interest (ROI) of the three OCT pullbacks were identified using landmarks of the coronary arteries. Quantitative analyses were performed by detection of lumen areas, diameters and volumes of the ROI were calculated. Differences in pullback lengths between the patients were compensated by calculating a normalized ROI [8]: Normalized ROI = [ROI volume/ no. slices in ROI

pullback] x median no. slices in ROIs of the population. Thrombus was visually scored as a mass protruding into the coronary vessel lumen [6].

The study was approved by the Ethics Committee of the University Medical Centre Groningen and all participants provided written informed consent.

Continuous variables are presented as means±standard deviation or as medians with interquartile range, ANOVA with repeated measures followed by Bonferroni's

Table 1. Baseline and histopathologic characteristics

	N = 30
Demographics	
Age, years	65.0±11.0
Male gender	24 (80)
Body mass index, kg/m2	29.0±4.7
Risk factors	
Diabetes	6 (20)
Hypertension	17 (57)
Hyperlipidemia	17 (57)
Current smoker	10 (33)
Family history	11 (37)
Previous CABG	1 (3)
Previous myocardial infarction	4 (13)
Previous PCI	4 (13)
Peak Troponin I, µg/L	1.07 (0.20–12.02)
Peak Troponin T, µg/L	0.14 (0.06-0.45)
Time from last complaints to PCI, days	4.4 (3.2–6.0)
Multivessel disease	12 (40)
Anterior infarction	16 (54)
Probability of in-hospital death (GRACE risk score)	
Low (<1%)	15 (50)
Intermediate (1-3%)	12 (40)
High (>3%)	3 (10)
Histopathologic analysis	
Effective thrombus aspiration	6 (20)
Thrombus	
White platelet thrombus	6 (20)
Red erythrocyte rich thrombus	0 (0)
Plaque	
Thrombus with plaque component	6 (100)
Thrombus without plaque component	0 (0)
Size	
None	2 (3)
Casts	22 (67)
Small (<0.5 mm)	3 (10)
Medium (0.5 - 2 mm)	3 (10)
Large (>2 mm)	0 (0)

The data are mean±SD, median, IQR, or numbers (%). CABG = coronary artery bypass graft; IQR = interquartile range; mm = millimeter; SD = standard deviation. Casts were defined as fragmental filter casts of loosely cohesive platelets which may not be defined as thrombus.

post-hoc test was used to compare continuous variables. Categorical variables are presented as numbers and percentages and were compared using the χ2 test. Statistical significance was defined as a two-sided p-value of <0.05.

Clinical and histopathological patient characteristics are presented in Table 1. Median time after the last complaints to PCI was 4.4 days. Histopathological analysis of the aspirated material showed aspiration of thrombus in only 20% of patients,

Table 2. Angiographic and OCT characteristics

	Before TA *N=30*	**After TA** *N=30*	**After stent** *N=30*	P-value
Invasive coronary angiography				
Thrombus	18 (60)	19 (63)	0 (0)	<0.001
TIMI Thrombus score				<0.001
0	12 (40)	11 (37)	30 (100)	
1	17 (57)	19 (63)	0 (0)	
2	1 (3)	0 (0)	0 (0)	
3/4/5	0 (0)	0 (0)	0 (0)	
TIMI flow grade				0.014
0/1	0 (0)	3 (10)	0 (0)	
2	15 (50)	11 (37)	6 (20)	
3	15 (50)	16 (53)	24 (80)	
Optical coherence tomography				
Thrombus	17/30 (57)	19/30 (63)	19/30 (63)	NS
Thrombus color				
White	11/17 (65)	13/19 (68)	NA	
Red	6/17 (35)	6/19 (32)	NA	
ROI length (mm)	24.23±9.27	24.23±9.27	24.23±9.27	
ROI volume (mm)	142.90±78.58	140.77±76.17	193.25±87.53†‡	<0.001*
Normalized ROI volume (mm3)	134.35±53.65	132.90±55.77	186.10±59.60†‡	<0.001*
Average area (mm2)	5.75±2.33	5.68±2.40	8.02±2.59 †‡	<0.001*
MLA (mm2)	1.69±1.04	1.81±1.12	5.66±2.62 †‡	<0.001*
Average diameter (mm)	2.59±0.52	2.56±0.55	3.14±0.53 †‡	<0.001*
Largest diameter (mm)	3.50±0.71	3.43±0.74	3.71±0.64 †‡	<0.001*
MLD (mm)	1.41±0.40	1.46±0.42	2.61±0.62 †‡	<0.001*

** 3-way comparison (before TA, after TA, after stent)*
† p<0.05 vs. before TA
‡ p<0.05 vs. after TA

The data are mean ± SD, or numbers (%). CROI = culprit region of interest; MLA = minimal lumen area; MLD = minimal lumen diameter; mm = millimeter; NA = not available; NS = not significant; ROI = region of interest; SD = standard deviation; TA = thrombus aspiration; TIMI = thrombus in myocardial infarction.

which were small amounts of fragmented atherosclerotic debris.

The angiographic and OCT data are provided in Table 2. TIMI thrombus score of 0 and 1 was observed in 29 patients before TA and in all patients after TA. Similarly, TIMI-flow did not improve after TA (TIMI-flow <3 in 15 patients before TA versus 16 patients after TA). Thrombus on OCT was observed in 17 patients, the majority being white thrombus. In 2 patients minimal amounts of white thrombus were induced by the guide wire or manipulation with the TA catheter. Moreover, minimal amounts of thrombus with plaque protrusion were observed after stent implantation in 19 patients (mean volume 1.67±1.69 mm^3). Mean volumes, diameters and areas of the ROIs were not significantly different before and after TA.

This study provides novel insight into TA in NSTEMI patients who underwent initial conservative therapy. First, although thrombus was observed on OCT in more than half of patients, the rate of retrieval of thrombus was low and only small amounts of atherothrombotic debris were found by histopathological analysis. Second, TA did not result in improved blood flow in the culprit coronary artery or in decrease of intracoronary thrombus burden on OCT. Revascularization results in better prognosis in NSTEMI patients [7]. However, embolization of thrombus into the microvasculature in patients with acute coronary syndrome may lead to microvascular obstruction which may be of prognostic significance [9,10]. Although TA in STEMI patients and less evidently in NSTEMI patients with early revascularization resulted in better TIMI flow, in the present study though, TIMI flow after TA did not improve. Also, no clear improvement of vessel lumen volumes and areas of the vessel were observed on OCT. It is worth mentioning though, that with continuous improvements of the device technology an increased rate of effective TA may be expected with a new generation TA catheters also in NSTEMI patients with less thrombus load.

Several study limitations should be mentioned. First, the applicable guidelines at the start of the study recommended early catheterization only in NSTEMI patients with features of high risk, whereas current guidelines recommend PCI within 72 hours in all patients [7]. Second, this pilot study had a limited sample size and was designed only to investigate the principle of TA in patients with expectedly low thrombus burden. Finally, the effect of TA was assessed by measuring differences of the intraluminal volume between OCT runs since direct quantification of thrombus is limited with OCT.

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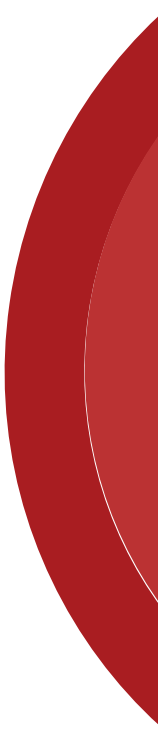

9

Summary and future perspectives

Nederlandse samenvatting

Curriculum Vitae

Dankwoord

Advances in treatment of patients with acute coronary syndrome have markedly improved cardiovascular outcomes over the last decades. Although revascularisation and pharmacological therapy provide benefit in terms of morbidity and mortality, still a large number of patients with coronary artery disease (CAD) will experience progression of the disease and/or occurrence of a coronary event. The ability to identify these patients may help to optimize the therapy and hence the clinical outcomes.

This thesis focuses on imaging techniques that may assist the identification of patients with CAD at higher risk for recurrent cardiovascular events and is divided in two parts. Part one explores the value of monitoring timelines of reperfusion and epicardial and myocardial patency after primary percutaneous coronary intervention (PCI) and its relationship to outcomes. Part two explores the value of non-invasive as well as invasive imaging methods in the evaluation and management of patients with acute coronary syndromes.

Monitoring of readily available variables in the timeline of onset, reperfusion and angiographic results of PCI in STEMI patients provide valuable information on patient outcomes. **Part 1** of this thesis investigates the impact of time from symptom onset to reperfusion and the time of symptom onset on angiographic as well as clinical outcomes.

Chapter 2 is a retrospective study in which we investigated the influence of time from symptom onset to reperfusion (ischemic time) in a cohort of 1383 STEMI patients undergoing PCI with thrombus aspiration and triple-anti platelet therapy. Myocardial reperfusion, as assessed by angiography (myocardial blush grade (MBG) of 3) and electrocardiography (ST-segment resolution >70%), significantly decreased after 5 hours of total ischemic time. Additionally total ischemic time was associated with mortality at 30 days, and mortality rates were nearly 3 times higher in patients with total ischemic time of more than 5 hours. However it should be taken into account that patients presenting after 5 hours were older and had more cardiovascular risk factors. The majority of STEMI patients were treated with PCI in the first 5 hours after symptom onset. We postulate a new concept of “first 5 golden hours” after the onset of symptoms, during which percutaneous intervention leads to significantly better myocardial reperfusion and clinical outcomes.

Chapter 3 explored the effect of time of onset of symptoms on myocardial infarction size and microvascular perfusion in relation to outcomes in a cohort of 6970 STEMI patients treated by PCI. The onset of symptoms is not uniform around the 24-hour clock with a peak incidence in the morning. Myocardial perfusion following PCI was not different during the day. Patients with onset of STEMI between 00:00 – 06:00

have longer ischemic time and larger myocardial infarction size. However mortality at 30 days was lower in these patients. In particular we observed that patients with low MBG presenting in the early morning hours have a better prognosis, suggesting a circadian relation of myocardial perfusion and infarct size with outcome.

In **part 2** of this thesis we focus on the use of non-invasive and invasive imaging methods that may be helpful in guiding cardiac interventions.**Chapter 4** is a review article, in which we provide an overview of current applications of the non-invasive imaging modality cardiac computed tomography (CT). It is discussed in which areas cardiac CT may replace invasive imaging techniques and areas in which cardiac CT may be useful in guiding cardiac interventions. Cardiac CT angiography provides useful anatomical information for planning of percutaneous coronary procedures, transcatheter valve replacements and catheter ablation of atrial fibrillation. More importantly coronary CT angiography is the only currently available clinically used non-invasive method capable of identifying coronary atherosclerotic plaques. Coronary CT angiography may be particularly useful in excluding coronary artery disease in patients with low and intermediate pre-test likelihood for CAD, thereby reducing the number of diagnostic invasive catheterizations and associated costs. Although valuable efforts have been attempted, non-invasive characterization of coronary artery plaques may currently not be used in prediction or diagnosing of acute coronary syndromes.

The ability of CT angiography for the detection and extent of CAD was used to explore the presence and characteristics of atherosclerotic plaque in patients presenting with acute myocardial infarction (MI) with normal coronary arteries on invasive coronary angiography (ICA) in **chapter 5**. In 30 patients with acute MI, coronary CT angiography was performed within 3 days after ICA. These patients were mainly women and the majority (80 %) of patients with a completely normal ICA showed no plaques on coronary CT angiography either. The rest of the patients had minimal, non-obstructive atherosclerosis in one coronary segment only. Thus the diagnosis of MI due to plaque rupture in these patients remains questionable. It would be reasonable to extend the diagnostic evaluations in these patients, e.g. with cardiac magnetic resonance imaging, because of the great implications the MI diagnosis has on these patients (psychological, secondary prevention, pharmaceutical treatment, insurance questions, health economy etc.).

Inflammatory and immunological events are considered to play a central role in initiation and progression of atherosclerotic plaques. Indeed, elevations in soluble markers of inflammation as well as changes in leukocyte subset distribution are frequently reported in patients with CAD. In **chapter 6** the relation of circulating immune-inflammatory markers, which have been linked to the atherosclerotic disease

process, with plaque burden and morphology as assessed by coronary CT angiography was investigated. Amongst the tested circulating immune-inflammatory markers, neutrophil count and neutrophil/lymphocyte ratio showed significant correlation wit non-calcified plaque burden and non-calcified/total plaque ratio. These results highlight the potential utility of these cellular markers in the risk assessment and monitoring of patients with CAD.

In **chapter 7** the ability of coronary CT angiography to differentiate advanced state coronary atherosclerotic lesions was investigated. In 30 patients with non-ST-elevation myocardial infarction (NSTEMI) we performed non-invasive as well as invasive in vivo evaluation of the coronary artery culprit lesion, using coronary CT angiography, intravascular ultrasound (IVUS) and optical coherence tomography (OCT) as a standard of reference. Normal findings on CTA related to early lesions on OCT. Nevertheless, several plaque features on CTA and IVUS related to advanced plaques on OCT. Thus, differentiation of advanced plaque using qualitative plaque features may proof difficult in clinical practice in patients with NSTEMI.

During PCI distal microembolisation of atherosclerotic debris may lead to a decline of microvascular perfusion. Therefore, removal of such debris, using an aspiration device, has the potential to limit microvascular obstruction as a complication of PCI. In STEMI patients manual thrombus aspiration improves clinical outcome. In NSTEMI patients with generally lower thrombus burden use of thrombus aspiration may as well be feasible during early mechanical reperfusion. In **chapter 8** it was investigated if OCT guided thrombus aspiration could be feasible in patients with NSTEMI after initial conservative treatment. Thrombus was observed in more than half of the 30 included patients, but the rate of retrieval of thrombus was low with only small amounts of atherothrombotic debris found by histopathological analysis. Thrombus aspiration did not result in improvement of coronary blood flow nor did it decrease the amount of thrombus detected by OCT. Therefore a generalized use of thrombus aspiration in NSTEMI patients with very low thrombus burden may not be feasible.

Future perspectives

Cardiovascular imaging provides a complementary diagnostic assessment and evaluation in the treatment of patients with CAD. If translated clinically, it will enable identification of patients who might benefit from additional management to improve their outcomes. For example, easy to obtain angiographic variables may assist in reclassification of patients with low and high susceptibility for future events. A clinically useful imaging method needs to have a discriminative value over traditional risk factors in prediction of patients susceptible to future clinical events. Ideally, it is

able to identify the individual patient in whom additional treatment is needed.

It is the aim of ongoing research efforts to define imaging modalities that provide parameters that can reliably be integrated into clinical diagnostic and therapeutic decision making. It is of particular clinical interest to determine which coronary lesion needs to be treated in order to prevent potentially life-threatening events in the future [1-3]. From the PROSPECT trial we can appreciate that, although certain structural characteristics (morphology) of non-culprit coronary plaques are related to new events, prediction of future events is not accurate enough to identify the specific plaques that will lead to new adverse cardiovascular events [4]. A combined approach directed at both structural characteristics as well as functional biological processes may be more informative and may provide higher predictive value than a single approach. In the end, the ultimate goal of any imaging test is guidance of therapy aiming at improvement of the outcome of patients [5].

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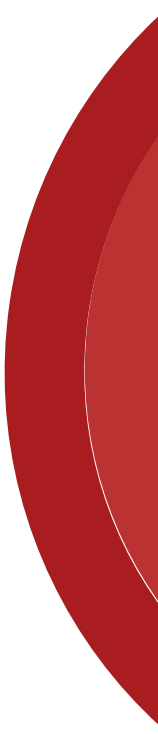


Hart- en vaatziekten zijn wereldwijd de belangrijkste doodsoorzaak. Ongeveer 40% van de sterfte door hart- en vaatziekten wordt veroorzaakt door kransvatlijden. Atherosclerose is de belangrijkste oorzaak van kransvatlijden. Het is een chronisch ziekte proces dat zich langzaam ontwikkeld waardoor het tientallen jaren kan duren voordat klachten optreden. De pathofysiologie van atherosclerose is complex; ontstekings- en immunologische processen spelen een belangrijke rol bij het ontstaan en ontwikkelen van atherosclerose.

Een acuut hartinfarct is een ernstig gevolg van atherosclerose van het kransvat. Wanneer een ruptuur van het atherosclerotisch weefsel plaats vindt, leidt dit tot een cascade van reacties met de vorming van een bloedstolsel als gevolg. Hierdoor raakt het kransvat gedeeltelijk of volledig afgesloten waardoor, als deze afsluiting te lang duurt, het hartspierweefsel onherstelbaar beschadigt.

In de behandeling van het acute hartinfarct zijn de afgelopen decennia grote vooruitgangen geboekt. Een belangrijke verbetering is het gebruik van percutane coronaire interventie (PCI, oftewel dotterbehandeling) en aanvullende medicamenteuze therapie. Ondanks deze verbeterde therapie zal door progressie van ziekte bij een groot deel van patiënten met atherosclerose opnieuw klachten ontstaan. Het vroegtijdig kunnen identificeren van deze patiënten kan helpen om de therapie en dus de klinische resultaten verder te optimaliseren.

Doordat atherosclerose een langzaam ziekte proces is dat in een laat stadium tot een hartinfarct leidt, bestaat de mogelijkheid om deze kwetsbare atherosclerotische plekken in het kransvat vroegtijdig op te sporen. Er zijn verschillende invasieve en niet-invasieve beeldvormende technieken die daarbij zouden kunnen helpen.

Dit proefschrift beschrijft de waarde van verschillende invasieve en niet-invasieve beeldvormende technieken voor de evaluatie en behandeling van patiënten met acute coronaire syndromen.

In het **eerste deel** van dit proefschrift worden verschillende parameters beschreven die de uitkomsten na een PCI behandeling beïnvloeden bij patiënten met ST-elevatie myocard infarct (STEMI).

Hoofdstuk 2 beschrijft de invloed van de ischemische tijd (tijdsduur tussen het ontstaan van klachten en behandeling) in een cohort van 1383 STEMI patiënten die een PCI ondergaan volgens de nieuwste standaard. De myocard perfusie (doorbloeding van de hartspier) na PCI, vastgesteld met coronair angiografie (myocardiale blush graad 3) en elektrocardiografie (ST-segment resolutie >70%) nam significant af na 5 uur totale ischemische tijd. Daarnaast was de totale ischemische tijd geassocieerd met sterfte in de eerste 30 dagen na PCI. De sterftecijfers waren bijna 3 keer hoger bij patiënten met een totale ischemische tijd van meer dan 5 uur. Deze patiënten

bleken echter ouder te zijn en meer cardiovasculaire risicofactoren te hebben. De meerderheid van STEMI patiënten werd in de eerste 5 uur na het ontstaan van de symptomen behandeld. PCI binnen de eerste 5 uren na ontstaan van klachten leidt tot significant betere myocardiale perfusie en klinische uitkomsten.

Hoofdstuk 3 onderzocht het effect van het moment van de dag waarop de klachten van het hartinfarct ontstaan en myocard perfusie na PCI in relatie tot klinische uitkomsten. Dit werd onderzocht in een cohort van 6970 STEMI patiënten die met PCI werden behandeld. Het tijdstip waarop klachten begonnen is ongelijk verdeeld over de dag, met de hoogste incidentie in de ochtend. Patiënten waarbij het hartinfarct tussen 0:00-06:00 uur begon, hebben een langere ischemische tijd en hebben meer schade aan de hartspier. Echter, het aantal sterfgevallen gemeten binnen 30 dagen na aanvang van klachten ligt bij deze patiënten lager ten opzichte van patiënten waarbij het hartinfarct op een ander moment van de dag begint. Opvallenderwijs vonden we dat patiënten met een slechte myocard perfusie die klachten kregen in de vroege ochtenduren een betere prognose hebben. Dit duidt op een 24-uurs patroon in de relatie tussen de myocard perfusie en de grootte van het hartinfarct met klinische uitkomsten.

In het **tweede deel** van dit proefschrift wordt de bruikbaarheid van invasieve en niet-invasieve beeldvormende technieken beschreven.

Hoofdstuk 4 geeft een overzicht van de huidige toepassing van de niet-invasieve beeldvormingstechniek cardiale computer tomografie (CT). Er wordt beschreven op welk gebied cardiale CT de invasieve beeldvormingstechnieken zou kunnen vervangen en hoe cardiale CT behulpzaam kan zijn bij cardiale percutane interventies. Cardiale CT-angiografie biedt nuttige anatomische informatie voor de planning van percutane procedures, zoals percutane hartklep vervangingen en katheter ablatie van boezemfibrilleren. Daarnaast is coronaire CT-angiografie momenteel de enig beschikbare klinisch gebruikte niet-invasieve methode die atherosclerose bij patiënten kan identificeren of uitsluiten. Het kan helpen het aantal invasieve diagnostische hartkatheterisaties en bijkomende kosten te verminderen. Er wordt veel onderzoek verricht naar de voorspellende waarde van de morfologie van atherosclerose, gemeten met CT-angiografie. Tot op heden is deze morfologische indeling nog te weinig specifiek om in de klinische praktijk te gebruiken voor het voorspellen van hartinfarcten.

Een klein deel van de patiënten die worden opgenomen in het ziekenhuis met een acuut hartinfarct hebben geen zichtbare afsluiting of vernauwing van het kransvat. In **hoofdstuk 5** werd CT-angiografie bij deze patiënten gebruikt om de aanwezigheid van atherosclerose op te sporen. Bij 30 patiënten met een acuut hartinfarct werd

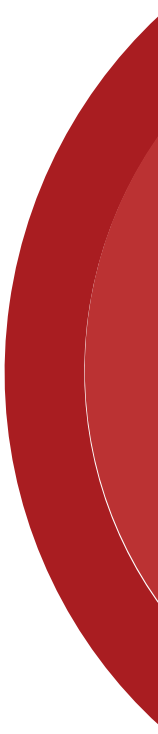
coronaire CT-angiografie uitgevoerd binnen 3 dagen na hartkatheterisatie. De groep patiënten betrof met name vrouwen en in de meerderheid (80%) werd, naast volledig normale bevindingen bij hartkatheterisatie, geen atherosclerose gevonden met behulp van coronaire CT-angiografie. De overige 20% van de patiënten had minimale niet-vernauwende atherosclerose in slechts één segment van het kransvat. Hierdoor wordt de diagnose van een hartinfarct als gevolg van atherosclerose bij deze patiënten twijfelachtig. Verdere diagnostiek is te overwegen bij deze groep patiënten, met bijvoorbeeld cardiale magnetische resonantie beeldvorming, vanwege de grote gevolgen die de diagnose hartinfarct heeft (psychisch, secundaire preventie, medicatie, verzekering, etc.).

Inflammatie (ontsteking) en immunologische (afweer) cellen spelen een centrale rol bij de ontwikkeling en progressie van atherosclerose. In **hoofdstuk 6** hebben we gekeken naar de relatie tussen in het bloed circulerende immuun-inflammatoire markers en de ernst en morfologie van atherosclerose. Atherosclerose werd beoordeeld met behulp van coronaire CT-angiografie. Van de onderzochte circulerende immuun-inflammatoire markers, toonde het aantal neutrofielen en de neutrofielen/lymfocyten verhouding een significante correlatie met de hoeveelheid niet-verkalkte atherosclerose en de verhouding niet-verkalkte atherosclerose/ totale hoeveelheid atherosclerose. Deze resultaten benadrukken het potentiële nut van deze cellulaire markers in de risico-evaluatie en monitoring van patiënten met atherosclerose.

In **hoofdstuk 7** is onderzocht of met behulp van coronaire CT-angiografie laat stadium coronaire atherosclerotische laesies kunnen worden onderscheiden. Bij 30 patiënten met een non-ST-elevatie myocard infarct (NSTEMI) werd zowel op invasieve als niet-invasieve wijze atherosclerose onderzocht. Coronaire CT-angiografie werd daarbij vergeleken met intravasculaire echografie (IVUS) en optische coherentie tomografie (OCT). OCT fungeerde daarbij als referentie standaard. Normale bevindingen op de CT correleerden met vroege laesies bij OCT. Verschillende morfologische kenmerken van atherosclerose gezien op CT en IVUS correleerden met laat stadium laesies op OCT. Hierdoor is het onderscheidend vermogen om laat stadium laesies te identificeren in de klinische praktijk lastig.

Tijdens het verrichten van PCI kan distale microembolisatie van het atherosclerotische debris optreden, wat kan leiden tot een verminderde doorbloeding van de microvasculatuur van de hartspier. Verwijdering van dit debris, met behulp van trombusaspiratie kan resulteren in minder micovasculaire obstructie en speelt hiermee een rol in het voorkomen van complicaties van een PCI. Bij STEMI patiënten verbetert de klinische uitkomst door het gebruik van een

dergelijke aspiratie katheter. Ook bij NSTEMI patiënten met een over het algemeen lagere trombus load, zou trombus aspiratie verricht kunnen worden. In **hoofdstuk 8** werd onderzocht of trombus aspiratie toepasbaar is bij patiënten met NSTEMI na initiële behandeling met medicijnen. OCT en histopathologie werden gebruikt om het effect van trombus aspiratie te evalueren. Trombus werd waargenomen bij meer dan de helft van de 30 geïncludeerde patiënten, maar de aspiratie bleek onvolledig aangezien slechts kleine hoeveelheden atherosclerotisch materiaal dat werd aangetroffen bij histopathologische analyse. Trombus aspiratie leidde niet tot verbetering van de coronaire perfusie en evenmin tot afname van de hoeveelheid trombus gedetecteerd door OCT. Daarom is gestandaardiseerd gebruik van trombus aspiratie in NSTEMI patiënten met een zeer lage trombus load niet zinvol.



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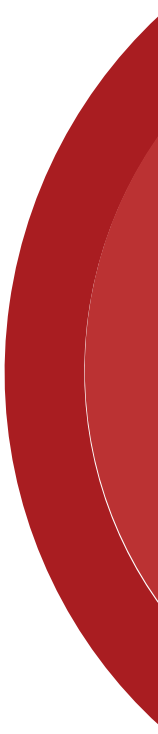
Wouter Wieringa werd op 16 maart 1984 geboren in Hallum, als oudste zoon van Gerben en Joke. Hij groeide met zijn twee zussen en broer op in Hallum. Op het Christelijk Gymnasium Beyers Naudé haalde hij in 2002 zijn diploma. Hierna begon hij met de studie geneeskunde aan de Rijksuniversiteit Groningen. In 2003 haalde hij zijn propedeuse. Bij de afdeling sportgeneeskunde deed hij zijn afstudeerscriptie. Wouter heeft zijn co-schappen in het St. Elisabeth Hospitaal, te Curaçao, en in het Deventer Ziekenhuis gelopen. Het keuze co-schap Cardiologie heeft hij in het Medisch Centrum Leeuwarden gedaan. Na het behalen van zijn artsexamen in de zomer van 2009 is Wouter in het Universitair Medisch Centrum Groningen begonnen als artsonderzoeker bij de afdeling Cardiologie. Aansluitend heeft hij onderzoek gedaan aan het Universitetssjukhuset Linköping, te Zweden. Na zijn promotie op 2 juni 2014 zal hij in het Universitair Medisch Centrum Groningen starten met de opleiding tot cardioloog.



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